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and the
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The Cytokine Odyssey 2001

Outrigger Wailea Resort
Maui, Hawaii
November 8-11, 2001

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Host Defense
and
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The Scientific Meeting, consisting of five plenary sessions, nine symposia, two poster sessions, a presidential address, a keynote address, two award lectures and a student award session was held in Maui, Hawaii, November 8-11, 2001. The scientific program chairs were Carl Ware and Tom Hamilton. Progress in the vital area of cytokines was described by major investigators in both basic science and clinical applications. Detailed summaries of these advances are discussed in the attached summary of the meeting, and abstracts of all papers delivered at the conference are enclosed in the abstract and program book. It was a very successful meeting.

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The Cytokine Odyssey 2001
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REPORT

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The Cytokine Odyssey 2001 was held at the Outrigger Wailea Resort in Maui, Hawaii. The meeting, jointly sponsored by the International Cytokine Society (9th Annual Meeting) and the Society of Leukocyte Biology (35th Annual Meeting), was organized by Carl Ware (Chair) from the La Jolla Institute for Allergy and Immunology (La Jolla, USA) and Thomas Hamilton (Co-Chair) from the Cleveland Clinic Foundation (Cleveland, USA). This international conference was designed to bring together leading investigators in molecular and cellular biology, physiology and genetics, interested in cytokines and cells of the immune system. This forum was aimed to assess the impact of this expanding science on new approaches to disease intervention [1].

Keywords: cytokines, chemokines, apoptosis, lymphocytes, dendritic cells, transcription, NF-AT, NF- κ B, innate immunity, autoimmune diseases

1. ICS Presidential Address, ICS Lifetime Achievement Awards and Keynote Address.

1.1 ICS Presidential Address: The saga of the balance ... or imbalance.

J-M. Dayer (University Hospital Geneva, Switzerland), President of the ICS retraced the history of the discovery of the role of cytokines in chronic inflammatory diseases, in particular rheumatoid arthritis (RA). The maintenance and function of healthy immune cells is the result of a balance between the production of cytokines (TNF α and IL-1) and their respective natural antagonists (TNF α inhibitory binding protein, TNF α BP and IL-1 receptor antagonist, IL-1-R α). In RA, monocytes and macrophages, which are activated by direct contact with stimulated T cells at the sites of inflammation, release massive amounts of pro-inflammatory cytokines (TNF α and IL-1) in the synovium. The cytokines trigger the production of proteolytic enzymes such as matrix metalloproteinases (MMPs) that ultimately leads to joint destruction. IL-1-Ra has been shown, in animal studies to be a specific and selective inhibitor of the IL-1 signaling pathway. IL-1-Ra therapy could constitute an important new approach to reduce inflammation and joint destruction in patients with RA. [2].

1.2. ICS Lifetime Achievement Award Lecture: Cytokine control of hematopoiesis and leukemia: from biology to the clinic.

L. Sachs (Weizmann Institute of Science, Rehovot, Israel) received the ICS Lifetime Achievement Award for his remarkable contribution in understanding how cytokines control normal hematopoiesis and leukemia. The maintenance, proliferation and differentiation of hematopoietic cells is regulated by a network of cytokine interactions which has positive as well as negative regulators. This cytokine network is a powerful tool in cancer therapy to reprogram malignant cells by inducing their differentiation with cytokines that regulate normal hematopoiesis or with antioxidants, calcium mobilizers or proteases inhibitors that mimic the effects of cytokines. Certain cytokines suppress apoptosis in normal and leukemic cells, which is a major problem in radiation and cytotoxic cancer therapies. These therapies can be improved by treatments that decrease the level of expression of cytokines controlling genes of the cellular apoptotic machinery [3].

1.3. Keynote Address: Cytokine regulation of genes of the apoptotic machinery.

D. Green (La Jolla Institute for Allergy and Immunology, La Jolla, USA) described the role of the cytokine Fas ligand (FasL) in the regulation of immune cells through induction of lymphocyte apoptosis. Activated T cells undergo FasL-mediated apoptosis. This process is mediated by upregulation of *FasL* gene expression in response to c-myc induction by activation of the T cell receptor. TGF β 1 can inhibit c-myc-mediated induction of *FasL* gene expression and subsequently apoptosis, a mechanism that is likely to play a role in antigen-dependent clonal expansion of T cells during immune response. D. Green also commented on the role of FasL-Fas interactions in neuronal cell death and

the potential therapeutic implications of modulating FasL activity in neurodegenerative diseases [4].

2. SLB Marie T. Bonazinga Award Lecture: TGF- β 1 in the treatment of experimental allergic encephalomyelitis (EAE).

G.S. Thorbecke (New York University School of Medicine, New York, USA) presented experimental data suggesting that T cells engineered to produce TGF β 1 are capable of down-regulating autoimmune and allergic reactions when delivered locally to the sites of inflammation. The studies were performed in several animal models of inflammatory diseases including EAE, collagen-induced arthritis (CIA) and asthma induced by inhalation of allergen. The protective effects of TGF β 1 are inhibited and the diseases relapse upon administration of neutralizing antibodies against TGF β 1 [5].

3. Cytokine signaling and effector function

3.1. Cytokine signaling and T cells.

3.1.1. Calcium/Calcineurin/NF-AT in T cell activation.

A. Rao (Harvard Medical School, Boston, USA) presented data demonstrating that the nuclear factor of activated T cells (NF-AT), which regulates signals from both calcium and phorbol esters signaling pathways, acts synergistically with other transcription factors such as AP-1 and NF- κ B to regulate the expression of diverse inducible genes. Gene expression profiling of D5Th1 cells stimulated with ionomycin alone or in combination with phorbol esters, revealed that NF-AT is the only transcription factor induced by calcium signaling alone. Using NF-AT mutant proteins unable to interact with AP-1 but retaining DNA binding and transcriptional activity, it was further demonstrated that there is a program of gene expression mediated by the calcium calcineurin NF-AT pathway, which is independent of the program induced by NF-AT – AP-1 cooperation [6].

G. Crabtree (Stanford University Medical School, Stanford, USA) developed a new concept that co-stimulation of T cells by CD3 and CD28 generates a double signal leading to activation of NF-AT. Gene expression profiling performed on lymphocytes stimulated with CD3 alone or with CD3 and CD28 indicates that CD28 stimulation induces a quantitative but not a qualitative increase in gene expression. CD28 stimulation activates the PI3 kinase pathway, which in turns blocks the activity of the glycogen synthase kinase-3 (GSK-3), responsive for rapid nuclear export of NF-AT. Simultaneously, CD28 enhances CD3-mediated dephosphorylation and nuclear import of NF-AT. This double signal maintains NF-AT in the nucleus allowing effective and sustained activation of target genes. Consistent with this model, transgenic mice expressing a nuclear form of NF-ATc1 in the thymus, develop a generalized autoimmunity. Their lymphocytes spontaneously express activation antigens and cytokines and are partially resistant to the immunosuppressive action of cyclosporin A [7].

3.1.2. Detection of IL-4 expression *in vivo*, using a bicistronic cytokine reporter.

R. Locksley (University of California San Francisco, San Francisco, USA) presented a very elegant approach to track effector T cells and assess immunity *in vivo*. R. Locksley's group engineered knockin mice expressing a bicistronic IL-4 reporter in which IL-4 is linked via a viral IRES element to the enhanced green fluorescent protein (EGFP). Mice subjected to *Nippostrongylus* infection develop a type2 immune response. CD4⁺ T cells expressing EGFP can be traced in immune tissues and maintain endogenous IL-4 expression. Furthermore, CD4⁺ T effector cells isolated from infected animals are fully capable of conferring immunoprotection against *Nippostrongylus* to immuno-deficient mice. Fluorescent reporter is therefore a valuable tool in evaluating tissue-specific cytokine expression *in vivo* and should be applicable to other cytokine genes [8].

3.1.3. CD4 gene silencing during T cell development.

D. Littman (New York University School of Medicine, New York, USA) elucidated the mechanism underlying CD4 transcriptional repression during T cell development. A silencer present in the *CD4* gene locus is sufficient for *CD4* transcriptional repression in thymocytes at early stages of differentiation, as well as in cell of the cytotoxic lineage (CD8⁺ T cells). Deletion of the *CD4* silencer before initiation of lineage specification in mice, results in *CD4* de-repression throughout thymocyte differentiation. In contrast, Cre-mediated deletion of the *CD4* silencer in mature CD8⁺ T cells shows no de-repression of the *CD4* gene. These results indicate that different mechanisms are likely to control *CD4* silencing in immature thymocytes versus CD8⁺ T cells. D Littman's group also identified the mammalian runt-related (Runx) protein 3 as one of the factors binding to the *CD4* silencer and responsible for *CD4* repression during T cell development [9].

3.2. Cytokine signaling and B cells.

3.2.1. Semaphorins

H. Kikutani (Research Institute for Microbial Diseases, Osaka University, Osaka, Japan) investigated the physiological function of semaphorins in the immune system by targeted disruption of semaphorins gene in mice. CD100^{-/-} mice show an impaired B cell activation characterized by a poor response of B cells to mitogenic signals and resistance to B cell receptor (BCR)-induced apoptosis. Dendritic cells (DCs) derived from CD100^{-/-} mice are defective in their ability to stimulate T cells indicating that CD100 is also involved in activation of DCs. CD100^{-/-} mice are also resistant to the development of EAE [10].

3.2.2. CD40 signaling in B lymphocytes.

G. Bishop (University of Iowa, Iowa, USA) presented experimental data suggesting that in B cells, CD40 signaling is initiated in membrane microdomains known as lipid rafts. Using confocal microscopy, she showed that CD40 activation also induces recruitment of Traf2 to the lipid rafts. Interaction between CD40 and Traf2 leads to activation of the downstream signaling pathways. Traf2 thereafter undergoes ubiquitination and

degradation. G. Bishop further reported that the oncoprotein latent membrane protein 1 (LMP1) of EBV mimics CD40 signaling in B cells. However, LMP1 delivers a more potent and sustained activation signal, which does not lead to degradation of Traf2 and may explain its oncogenic function [11].

3.3. Decoy receptors.

A. Mantovani (Mario Negri Institute, Milan Italy) illustrated the concept of decoy receptors as a general strategy used by immune cells to regulate inflammatory cytokines and chemokines. Decoy receptors recognize certain inflammatory cytokines (TNF α , IL-1 and IL-10) with high affinity and specificity and block the interaction between the cytokine and its effector receptor by sequestering the ligand. The first decoy receptor identified was the type II IL-1 receptor (IL-1IIR), which blocks IL-1 signaling. Decoy receptors have also been identified amongst members of the TNF and IL-10 families. Moreover, functionally uncoupled and non-signaling receptors acting as decoys have been identified amongst the chemokine receptors. Duffy/Darc and D6, two transmembrane chemokine receptors are structurally defective in inducing leukocyte migration and activation as they lack the DRY motif in their intracellular domain, which is required for ligand-dependent receptor activation. Duffy/Darc and D6 may therefore represent the first decoy receptors in the chemokine system. Decoy receptors represent an ideal therapeutic tool to inhibit specific cytokine and chemokine signaling pathways in inflammatory diseases [12].

3.4. Cytokine signaling and apoptosis.

3.4.1. Death domain-like modules in cell death and inflammation

J. Tschopp (University of Lausanne, Switzerland) commented on the identification of a new signaling pathway triggered by proteins with a pyrin domain (PYD) implicated in apoptosis and inflammation. The apoptotic machinery relies on the interaction between death domain receptor such as Fas and intracellular adaptor proteins containing one of the three protein interaction domains, death domain (DD), death effector domain (DED) and caspase activation and recruitment domain (CARD) involved in activation of the caspase-mediated apoptotic cascade. A fourth protein domain, the PYD domain, first identified in the familial Mediterranean fever protein, was found based on sequence similarity in a number of proteins involved in apoptosis and inflammation [13]. Tschopp's group identified a PYR protein that couples caspase 1 and activates it for cleavage of IL-1 β

3.4.2. Role of FADD in cytokine-mediated T cell activation.

S. Hedrick (University of California San Diego, La Jolla, USA) presented data supporting a role for the apoptotic signaling through the death domain adapter protein FADD in T cell activation. Transgenic mice expressing a truncated form of FADD lacking the caspase effector domain (FADDdd) placed under the control of the *lck* promoter show normal apoptosis during thymic negative selection and no lymphoproliferative disorders. However, these mice have a retarded thymocyte development and a reduced number of

peripheral mature T cells. A similar phenotype was observed in mice overexpressing CrmA, a caspase inhibitor downstream of FADD. Both transgenic lines were defective in T cell activation through the TCR and CD28. In addition, mature T cells were defective in their proliferative response to a subset of cytokines including IL-2 and IL-15 [14].

3.5. NF- κ B activation.

S. Ghosh (Yale University, Yale, USA) emphasized a major role of phosphorylation of the NF- κ B subunit p65 in regulating its transcriptional activity. Mice with a knockin mutation of serine 276 in p65, a site known to be phosphorylated by the catalytic subunit of protein kinase A, die at embryonic day (ED) 15.5 from massive liver apoptosis. This phenotype is essentially identical to that of the p65-deficient mice. The mutated form of p65 (m/mp65) still associates with I κ B α and undergoes nuclear translocation upon cytokine-induced degradation of I κ B α . However, m/mp65 is impaired in *in vivo* DNA binding and transcriptional activity. S. Ghosh proposed a model of transcriptional activation by NF- κ B. In non-stimulated cells, p50 homodimers associate with the histone deacetylase repressor HDCA1 and repress transcription. Upon stimulation, I κ B α degradation and p65 phosphorylation are required to generate fully active NF- κ B dimers that translocate to the nucleus, recruit CBP/p300, and displace the repressor from κ B binding sites allowing transcription to turn on [15].

4. From Innate to Adaptive Immunity.

4.1. NK cells react to cellular stress.

L Lanier (University of California San Francisco, San Francisco, USA) commented on the role of the activating receptor NKG2D in immune recognition and response to abnormal, transformed or pathogen-infected cells. NKG2D is expressed on most natural killer (NK) cells as well as CD8⁺ T cells and activated macrophages. In human, NKG2D recognizes the MHC class I like molecules, MICA and MICB as well as ULBP proteins, which are homologs of the mouse retinoic acid early inducible (RAE-1) proteins. *MIC* and *RAE1* genes are expressed during development but not in normal adult tissues. These genes are induced by cellular stress and are often expressed in malignant cells. Ectopic expression of MIC or RAE-1 on tumor cells leads to their killing by NK cells. These studies identify NKG2D as a major activating receptor of NK cells, involved in protection against infection and possibly malignancies [16].

4.2. The immunological synapse.

M. Dustin (New York University School of Medicine, New York, USA) proposed a model in which effective immune response occurs by formation of an immunological synapse, a supramolecular structure at the interface between the T cell and the antigen-presenting cell (APC). Two-dimensional supported planar bilayers containing fluorescently-labelled adhesion molecules and MHC peptide complexes can be used to

mimic T cell stimulation by APCs and to visualize the formation and stability of the immunological synapse, to study the dynamic interactions between these two cells as well as to analyse the influence of co-stimulation and chemokine gradients on T cell activation and migration [17].

5. Innate Immunity.

5.1. Toll-like receptor activation by Mycobacteria.

M. Fenton (Boston University School of Medicine, Boston, USA) commented on the role of Toll-like receptors (TLRs) in mediating innate immune response to mycobacterial infection. Mycobacteria activate macrophages via TLR-dependent and TLR-independent pathways. Activation via TLR2 and TLR4 induced expression of pro-inflammatory cytokines and chemokines and generate a potent Th1-type immune response. This response is dramatically impaired in mice deficient in TLR2 and TLR4 as well as in the C3H/HeJ mouse strain that expressed a no-functional mutant TLR4. Mycobacteria however also induce production of nitric oxide by activated macrophages, by activation of a second, TLR-independent pathway [18].

5.2. Dendritic cell response to viral challenges.

C. Biron (Brown University, Providence, USA) studied the activation of dendritic cells (DCs) *in vivo* upon viral challenges of mice with lymphocytic choriomeningitis virus (LCMV) or murine cytomegalovirus (MCMV). Both viruses induce production of type I interferons ($\text{IFN}\alpha/\beta$) by DCs but only MCMV also induces IL-12 production. $\text{IFN}\alpha/\beta$ contributes to the activation and proliferation of NK cells, to the inhibition of IL-12 production and to the induction of IL-15 expression, a STAT1-dependent signaling pathway. IL-12 induction during MCMV infection results in production of $\text{IFN}\gamma$ by NK cells, a STAT4-dependent pathway. Type I IFNs are therefore capable of modulating immune response to viral pathogens by simultaneously activating immune responses and inhibiting the innate cytokine-mediated response.

6. Chemokines.

6.1. DCs link inflammation to immunity.

K. Matsushima (Tokyo University School of Medicine, Tochigi, Japan) presented experimental data demonstrating a pivotal role for inflammation-activated DCs in establishing a Th1-mediated immune response. Using a bacteria-induced liver injury (granulomatous) model in mice, K. Matsushima showed that DCs are recruited to the hepatic granulomas and further move to the portal area and the hepatic lymph nodes (LNs). These successive steps of DCs migration from the bloodstream to the hepatic LNs are controlled by different chemokines, mainly MIP-1a/CCL3 and SLC/CC21. Mature DCs thereafter interact with nonpolarized T cells to form DC-Th1 cell clusters, a process that is controlled by the chemokine CXCL10.

6.2. Chemokines as systemic organizers of the immune response.

M. Lipp (Max-Delbrück-Center for Molecular Medicine, Berlin, Germany) commented on the role of chemokines and their receptors as major regulators involved in the functional organization of secondary lymphoid organs by controlling migration and homing of T cells, B cells and DCs. CXC chemokine receptor-5 (CXCR5) is required for B cell homing to B follicles. Mice deficient in CXCR5 lack Peyer's patches (PPs) and show a severely impaired organization of splenic primary follicles. The chemokine receptor CCR7 is required to rapidly initiate the primary immune response by bringing together DCs and lymphocytes into secondary lymphoid organs. These chemokine receptors may therefore play a role in Hodgkin's disease (HD). M. Lipp showed that HD cell lines have a limited expression of the chemokine and chemokine receptor repertoire. The classical form of HD shows high expression level of CCR7 and CXCR4, but not of CXCR5, and express the B lymphocyte chemokine (BCL/CXCL13), a ligand of CXCR5. M. Lipp also reported that the novel synthetic immunosuppressant FT720 increases homing of B lymphocytes in LNs and PPs in CCR7-deficient mice [19].

6.3. Chemokine polymorphism and human diseases.

P. Murphy (National Institute of Health, Bethesda, USA) analyzed the possible association between genetic polymorphisms affecting the function or expression of chemokines and chemokine receptors and the development of certain human disease. A polymorphism in the chemokine receptor CX3CR1 that affects receptor function is an independent risk factor for atherosclerotic coronary artery disease. Genetic variations in the chemokine receptors CCR2 and CCR5 are also markers for acute rejection in renal transplantation [20].

6.4. CXCL1 and melanomas.

A Richmond (Vanderbilt University School of Medicine, Nashville, USA) reported that the melanoma growth stimulatory activity-regulated protein CXCL1 is often constitutively expressed in melanomas and squamous cell carcinomas as a result of increased transcription of the *CXCL1* gene as well as increased *CXCL1* mRNA stability. The transcription of *CXCL1* is normally repressed by binding of the CCAAT displacement protein (CDP) to a DNA element (IUR) in the *CXCL1* promoter. The IUR is directly upstream of the NF- κ B site and is required for basal and cytokine-induced transcription of the *CXCL1* gene. The IUR also binds the poly (ADP-ribose) polymerase (PARP1), which possibly acts as co-activator of *CXCL1* transcription. A. Richmond proposed that in melanomas, the balance between repression of *CXCL1* transcription by CDP and activation by PARP1 and other transcription factors is impaired and lead to constitutive transcription of the *CXCL1* gene [21].

7. Clinical Impact of Cytokines.

7.1. Cytokines and APCs in EAE.

N. Ruddle (Yale University School of Medicine, Connecticut, USA) investigated the cellular origin of cytokines produced in the central nervous system (CNS) in myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE), a murine model for multiple sclerosis. During EAE, activated CD4⁺ T cells invade the CNS and secrete Th1 cytokines such as IFN γ and TNF α . Activation of these T cells occurs via interaction with CNS APCs such as infiltrating macrophages, resident microglia and CNS DCs. CNS APCs however also produce nitric oxide (NO) that inhibits the proliferation of T cells, thereby confining the autoreactive T cells to the CNS. CNS APCs, though essential for the initiation of the disease, also play a down-regulatory role. N. Ruddle also pointed out that B cells are not required for EAE-induced by rodent MOG but well for EAE-induced by human MOG. This switch in the pathogenic mechanism is due to a one amino acid sequence difference between human and rodent MOG that confers to human MOG a very poor ability to stimulate T cells [22].

7.2. Cancer immunoediting by IFN γ and lymphocytes.

R. Schreiber (Washington University School of Medicine, St Louis, USA) commented on the critical role for IFN γ signaling and lymphocytes in protecting against primary tumor development and in enhancing tumor cell immunogenicity, a process that R. Schreiber defined as "cancer immunoediting". IFN γ insensitive mice that lack either the IFN γ receptor or STAT1 are more susceptible to develop spontaneous or carcinogen-induced tumors. Similar results were observed in mice with a targeted disruption of the recombination-activating gene-2 (RAG2) that is expressed only in lymphocytes. Mice deficient in RAG2 and STAT1 develop an even wider array of spontaneous tumors. Collectively, these results indicate that IFN γ and lymphocytes collaborate to protect immunocompetent mice against tumor development and functions as an intrinsic tumor-suppressor system [23].

7.3. TRAIL in protection against tumors.

K. Okumura (Juntendo University School of Medicine, Tokyo, Japan) provided experimental evidence for a physiological function of the tumor necrosis-related apoptosis-inducing ligand (TRAIL) as a tumor suppressor. TRAIL is constitutively expressed on murine NK cells in the liver and is responsible for their cytotoxicity against TRAIL-sensitive tumors *in vitro* along with perforin and Fas ligand. More importantly, administration of neutralizing monoclonal antibody against TRAIL promotes tumor development in mice subcutaneously inoculated with TRAIL-sensitive tumor cells. The anti-metastatic effect of TRAIL is mediated by NK cells, and required production of IFN γ . These findings provide the first evidence for a role of TRAIL in tumor surveillance [24].

7.4. TWEAK and angiogenesis.

S. Wiley (Immunex Corp., Seattle, USA) presented data supporting a major role for TWEAK, a member of the TNF ligand family in proliferation of endothelial cells.

vasculature formation and induction of angiogenesis. Antibody crosslinking of the TWEAK receptor stimulates endothelial cell proliferation, a process that does not involve upregulation of VEGF. Pellets containing recombinant TWEAK induce a strong angiogenic response with neovascularization *in vivo* when implanted in rat corneas, a classical model for angiogenesis. Using the rat carotid balloon injury, a model to study vascular endothelial regeneration, S. Wiley showed that TWEAK receptor transcription is induced in endothelial cells of denuded rat carotid artery. These results support a direct but not restricted role for TWEAK signaling in endothelial cell proliferation and angiogenesis [25].

7.4. EDAR signaling and ectodermal dysplasia.

J. Zonana (Oregon Health Sciences University, Portland, USA) described the genetic basis for the human developmental disorder X-linked hypohidrotic ectodermal dysplasia (XLHED), a disorder characterized by impaired development of hair, teeth and eccrine sweat glands. This inherited disease is linked to loss of function of proteins involved in the signaling pathway triggered by the epithelial morphogen ectodysplasin-A (EDA), a member of the TNF-ligand superfamily. Signaling through EDA is mediated by two distinct receptors, EDAR and XEDAR and involves activation of the I κ B kinase complex (IKK) and NF- κ B. Mutations leading to XLHED have been identified in different functional domains of EDA or of its receptors, which all lead to impaired ligand-receptor interaction. Several mutations in the zinc finger domain of IKK γ , the regulatory subunit of IKK, are also linked to the development of XLHED. A death domain protein (EDARAdd) with similarity to MyD88 was identified as part of the EDAR signaling pathway. Mutations in this adaptor protein cause XLHED [26].

7.5. BlyS, APRIL and B cell development

J. Gross (Zymogenetics Inc, Seattle, USA) discussed the distinct biological roles of the B lymphocyte stimulator (BlyS/BAFF) and APRIL (a proliferation-inducing ligand), two related members of the TNF ligand superfamily involved in B cell development. Both BlyS and APRIL bind the cell surface receptor B cell maturation antigen (BCMA) and transmembrane activator and CAML interactor (TACI). BlyS also signals via the B cell specific receptor BAFFR. Mice treated with TACI-Ig or TACI-Ig transgenic mice present a complete blockage in B cell maturation. TACI-Ig also suppresses the production of collagen specific antibodies and inhibits the progression of the disease in collagen-induced arthritis, a mouse model for rheumatoid arthritis. TACI-Ig acts as a soluble decoy receptor by blocking both BlyS's and APRIL's biological activities and therefore could be used as therapeutic agent in the treatment of autoimmune diseases. Studies in BlyS-deficient mice indicate that BlyS is required for pre-immune B cell survival and development [27].

7.6. Manipulation the lymphotoxin system

J. Gommerman (Biogen, Cambridge MA, USA) illustrated the importance of the lymphotoxin-beta (LT β) signaling pathway in the maintenance and organization of the immune function in secondary lymphoid organs. In the spleen, LT β is required to maintain the number and function of the follicular dendritic cells. LT β controls the splenic marginal zone microenvironment necessary for generation of autoreactive B cells. While initial T cell priming and expansion does not rely on LT β , the migration of effector T cells to their sites of action is controlled by LT β -dependent chemokine production. In vivo studies using a soluble form of the LT β receptor (LT β R-Ig) acting as an inhibitor of the LT β signaling pathway, demonstrated that modulation of the LT β signaling pathway may be a novel approach in the treatment of immune diseases [28].

8. Cytokines are the essential mediators of immunity and inflammation and now recognized as potential candidates for therapeutic intervention.. Several major families of cytokines exist including the interferons, chemokines, tumor necrosis factors, interleukins and transforming growth factors, and with literally dozens of individual cytokines within some families, this field offers a treasure trove of targets to pick from. Which is the major conundrum of pharmaceutical development. Many of the talks provided significant new insight into which cytokines are hot for development and those that are emerging as potential targets. The expression of cytokines in the extracellular milieu provides ready access to therapeutic antibodies and decoy receptors, which have proven their utility in human disease. However specificity and redundancy of some cytokine systems has turned some to investigate intracellular signaling pathways as targets. Many cytokines activate common intracellular signaling pathways, such as NF κ B, which may be a more direct and common route to block inflammation. Basic research, as displayed at this conference to the 450 participants, is revealing the multifaceted biology and physiology of these molecules, much more complexity than initially conceived. Effective targeting of cytokines will require a detailed knowledge of their cellular and physiological effects, thus contacts among the cellular biologists and cytokinologists, especially in beautiful Maui, should continue to provide productive and rewarding understanding of disease pathogenesis and therapeutic interventions.

9. Future meetings

The 2002 joint meeting of the International Cytokine Society (ICS), the International Society for Interferon and Cytokine Research (ISICR), the European Cytokine Society (ECS) and the Society of Leukocyte Biology (SLB) will be held at the Lingotto Congress Center, Torino, Italy on October 6 - 11, 2002 and is organized by S. Landolfo, G. Garotta, P. Ghezzi, A. Mantovani, S. Schwarzmeier and G. Trinchieri.

Website: <http://www.marionegri.it/cyto2001>; Email: cyto2001@marionegri.it

A related meeting focused on tumor necrosis factor-related cytokines, the TNF Superfamily 2002, will be held on October 30-November 2, 2002 in San Diego

California. This is the 9th International Congress on TNF-related Cytokines. For more information visit the website: <http://meetings.liai.org/tnf>

10. References

1. Cellular and Molecular Mechanisms of Host Defense and Inflammation. *Journal of Leukocyte Biology* 2001, **Suppl. S**.
2. Dayer, JM, Feige U, Edwards CK, 3rd, Burger D. Anti-interleukin-1 therapy in rheumatic diseases. *Current Opinion in Rheumatology* 2001, **13**, 170-6.
3. Lotem, J, Sachs L. Cytokines as suppressors of apoptosis. *Apoptosis* 1999, **4**, 187-96.
4. Pinkoski, MJ, Green DR. Fas ligand, death gene. *Cell Death and Differentiation* 1999, **6**, 1174-81.
5. Thorbecke, GJ, Umetsu DT, deKruyff RH, Hansen G, Chen LZ, Hochwald GM. When engineered to produce latent TGF-beta1, antigen specific T cells down regulate Th1 cell-mediated autoimmune and Th2 cell-mediated allergic inflammatory processes. *Cytokine and Growth Factor Reviews* 2000, **11**, 89-96.
6. Macián, F, López-Rodríguez C, Rao A. Partners in transcription: NFAT and AP-1. *Oncogene* 2001, **20**, 2476-89.
7. Neilson, J, Stankunas K, Crabtree GR. Monitoring the duration of antigen-receptor occupancy by calcineurin/glycogen-synthase-kinase-3 control of NF-AT nuclear shuttling. *Current Opinion in Immunology* 2001, **13**, 346-50.
8. Mohrs, M, Shinkai K, Mohrs K, Locksley RM. Analysis of type 2 immunity in vivo with a bicistronic IL-4 reporter. *Immunity* 2001, **15**, 303-11.
9. Zhou, Y-R, Sunshine M-J, Taniuchi I, Hatam F, Killeen N, Littman DR. Epigenetic silencing of CD4 in T cells committed to the cytotoxic lineage. *Nature Genetics* 2001, **29**, 332-336.
10. Watanabe, C, Kumanogoh A, Shi W, Suzuki K, Yamada S, Okabe M, Yoshida K, Kikutani H. Enhanced immune responses in transgenic mice expressing truncated form of the lymphocyte semaphorin CD100. *The Journal of Immunology* 2001, **167**, 4321-4328.
11. Hostager, BS, Catlett IM, Bishop GA. Recruitment of CD40 and tumor necrosis factor receptor-associated factors 2 and 3 to membrane microdomains during CD40 signaling. *Journal of Biological Chemistry* 2000, **275**, 15392-8.
12. Mantovani, A, Locati M, Vecchi A, Sozzani S, Allavena P. Decoy receptors: a strategy to regulate inflammatory cytokines and chemokines. *Trends Immunol* 2001, **22**, 328-36.
13. Martinon, F, Hofmann K, Tschopp J. The pyrin domain: a possible member of the death domain-fold family implicated in apoptosis and inflammation. *Current Biology* 2001, **10**, R118-20.
14. Walsh, CM, Wen BG, Chinnaiyan AM, O'Rourke K, Dixit VM, Hedrick SM. A role for FADD in T cell activation and development. *Immunity* 1998, **8**, 439-49.

15. Zhong, H, SuYang H, Erdjument-Bromage H, Tempst P, Ghosh S. The transcriptional activity of NF-kappaB is regulated by the IkappaB-associated PKAc subunit through a cyclic AMP-independent mechanism. *Cell* 1997, **89**, 413-24.
16. Cerwenka, A, Bakker AB, McClanahan T, Wagner J, Wu J, Phillips JH, Lanier LL. Retinoic acid early inducible genes define a ligand family for the activating NKG2D receptor in mice. *Immunity* 2000, **12**, 721-7.
17. Bromley, SK, Burack WR, Johnson KG, Somersalo K, Sims TN, Sumen C, Davis MM, Shaw AS, Allen PM, Dustin ML. The immunological synapse. *Annual Review of Immunology* 2001, **19**, 375-96.
18. Golenbock, DT, Fenton MJ. Extolling the diversity of bacterial endotoxins. *Nature Immunology* 2001, **2**, 286-8.
19. Lipp, M, Burgstahler R, Müller G, Pevzner V, Kremmer E, Wolf E, Förster R. Functional organization of secondary lymphoid organs by the chemokine system. *Current Topics in Microbiology and Immunology* 2000, **251**, 173-9.
20. McDermott, DH, Halcox JP, Schenke WH, Waclawiw MA, Merrell MN, Epstein N, Quyyumi AA, Murphy PM. Association between polymorphism in the chemokine receptor CX3CR1 and coronary vascular endothelial dysfunction and atherosclerosis. [Comment In: *Circ Res.* 2001 Aug 31;89(5):376-7 UI: 21423292]. *Circulation Research* 2001, **89**, 401-7.
21. Nirodi, C, Hart J, Dhawan P, Moon N-S, Nepveu A, Richmond A. The role of CDP in the negative regulation of CXCL1. *The Journal of Biological Chemistry* 2001, **276**, 26122-26131.
22. Juedes, AE, Ruddle NH. Resident and infiltrating central nervous system APCs regulate the emergence and resolution of experimental autoimmune encephalomyelitis. *Journal of Immunology* 2001, **166**, 5168-75.
23. Shankaran, V, Ikeda H, Bruce AT, White JM, Swanson PE, Old LJ, Schreiber RD. IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature* 2001, **410**, 1107-11.
24. Takeda, K, Hayakawa Y, Smyth MJ, Kayagaki N, Yamaguchi N, Kakuta S, Iwakura Y, Yagita H, Okumura K. Involvement of tumor necrosis factor-related apoptosis-inducing ligand in surveillance of tumor metastasis by liver natural killer cells. *Nature Medicine* 2001, **7**, 94-100.
25. Lynch, CN, Wang YC, Lund JK, Chen Y-W, Leal JA, Wiley SR. TWEAK induces angiogenesis and proliferation of endothelial cells. *The Journal of Biological Chemistry* 1999, **274**, 8455-59.
26. Gross, JA, Dillon SR, Mudri S, Johnston J, Littau A, Roque R, Rixon M, Schou O, Foley KP, Haugen H, McMillen S, Waggle K, Schreckhise RW, Shoemaker K, Vu T, Moore M, Grossman A, Clegg CH. TACI-Ig neutralizes molecules critical for B cell development and autoimmune disease. impaired B cell maturation in mice lacking BLyS. *Immunity* 2001, **15**, 289-302.

A Joint Meeting of the
SOCIETY FOR LEUKOCYTE BIOLOGY
and the
INTERNATIONAL CYTOKINE SOCIETY

The Cytokine Odyssey 2001

Program
Abstracts
Member Directory

Outrigger Wailea Resort
Maui, Hawaii
November 8-11, 2001

ACKNOWLEDGMENTS

*The Society for Leukocyte Biology and the International Cytokine Society
gratefully acknowledge:*

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JOURNAL OF LEUKOCYTE BIOLOGY®

An Official Publication of the Society for Leukocyte Biology

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KEY TO PROGRAM

First number is the Program number, which corresponds to the abstracts (see pages 19-102). The second number (boldface) is the Poster Board number.

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GENERAL INFORMATION

REGISTRATION

Hours

Meeting Registration Desk is located in the

Aulani Foyer of the Outrigger Wailea Resort.

Thursday, November 8.....1 PM - 5 PM

Friday, November 9.....8 AM - 5 PM

Saturday, November 1.....8 AM - 5 PM

Sunday, November 11.....8 AM -12 PM

Fees

On site

Participant (Acad/Gov) \$490.00

Participant (Industry) \$790.00

Participant (Student) \$335.00

Accompanying Persons \$230.00

Institutional identification is required for academic, government, and student rates.

The registration fee for all meeting participants includes scientific sessions, exhibits, opening reception, awards banquet, continental breakfasts, refreshment breaks, and conference materials. The registration form may be photocopied if additional copies are needed. Attendees may pick up the registration confirmation, Congress Program /Abstract Book, badges, and other meeting materials at the Registration Desk.

Registration Categories

For registration in the **Academic/Government** category, please provide institutional identification. **Student Registration** is open, at a reduced price, to any fully matriculated student working toward a degree in one of the biomedical sciences. Registrants in this category must have a department head or research advisor certify their eligibility on this form. If registering at the meeting, bring a student ID card or letter signed by your department head. Those without proper student credentials must pay the full registration fee.

Spouse/Guest Registration (accompanying person) permits a guest or spouse and other nonscientist family members of the participant to attend social functions at cost. This is a social registration fee, which includes the opening reception, continental breakfasts, and admission to the Awards Banquet; it does **not** include meeting materials or admission to the scientific sessions.

HOUSING

Outrigger Wailea Resort is located on the south coast of Maui, Hawaii, about 25 minutes from Kahului Airport. Meeting participants may take advantage of the special conference rates listed below. Call (800) 367-2960 or (808) 879-1922 to make reservations. Requests may also be faxed to (808) 879-7658. Mention that you will be attending the Cytokine Conference.

Room Rate

Standard/Garden View \$144.00

Ocean View \$158.00

Ocean Front \$174.00

Assignment of the type of guest room depends on availability at the time of booking.

All rates are based on single or double occupancy, per room, per day. A Transient Accommodation Tax (currently 7.25%) and Hawaii State General Excise Tax (currently 4.166%) will be added to all quoted room rates; taxes are subject to change. The hotel requires one night's prepayment of the room and tax for guest room reservations. Deposits are forfeited if cancellations are received within seven (7) days prior to arrival. **If you are a no-show or cancel within 72 hours of your planned arrival, you will be responsible for the cost of the guest room and tax for the entire length of your reservation.**

SLB-SPONSORED AWARDS

- Presidential Student Awards in Research
- Student Travel Awards

Competitors must be SLB members.

ICS-SPONSORED AWARDS

- Honorary Lifetime Membership
- Young Investigator Award
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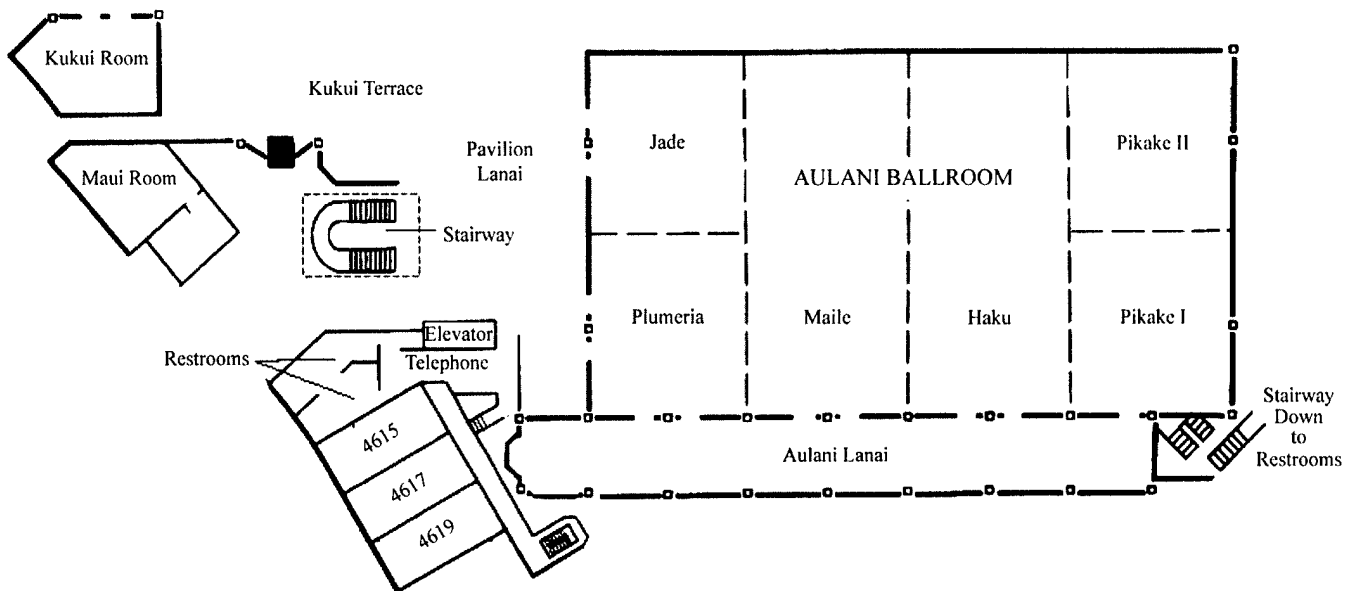
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Continuing Medical Education (CME)

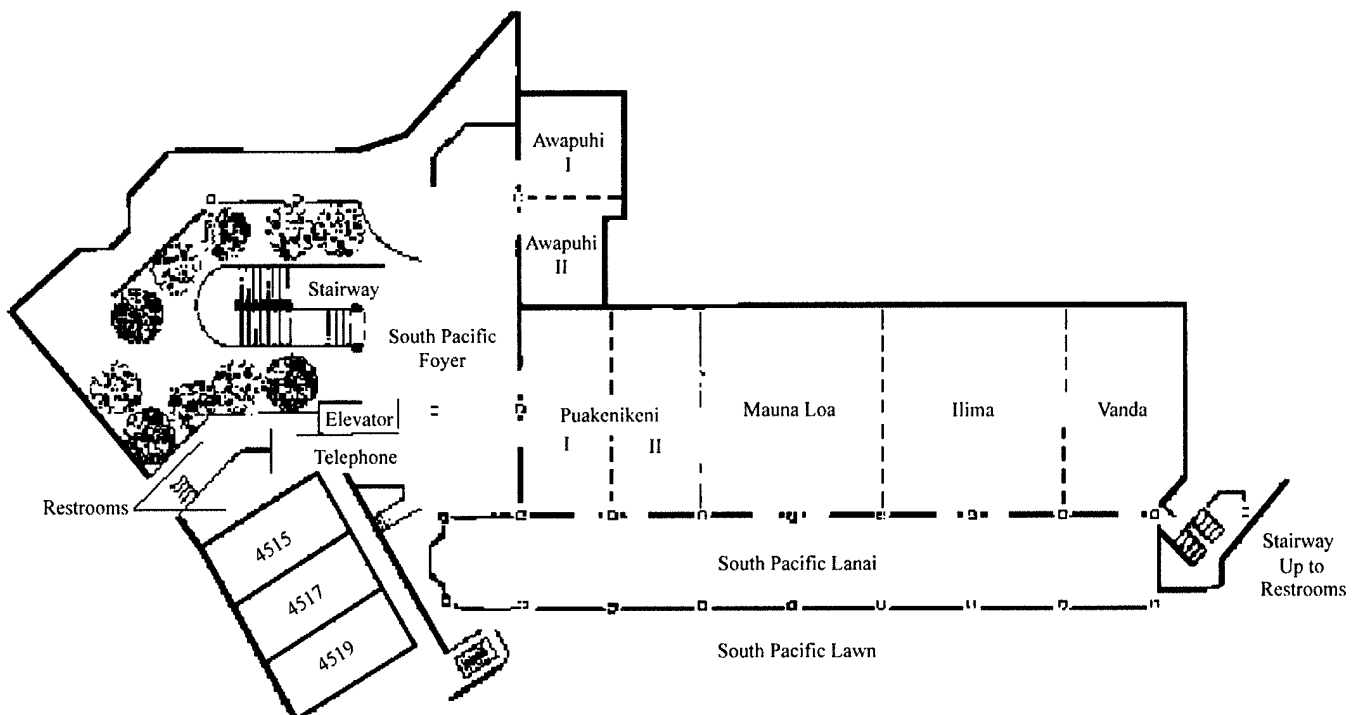
CME credits will not be awarded.

Outrigger Wailea Resort

Aulani Ballroom — Lobby Level South Conference Pavilion



South Pacific Ballroom — Conference Level South Conference Pavilion



EXHIBITORS

Journal of Leukocyte Biology

As the official publication of the Society for Leukocyte Biology, *JLB* publishes original research dealing with the cell and biology, developmental biology and functions of granulocytes, mononuclear phagocytes, lymphocytes, NK cells, and other cells involved in host defense. The journal also publishes review articles and occasional issues on one theme. Online submission of manuscripts is now available.

AWARDS COMPETITION

Presidential Awards

Predoctoral students and postdoctoral trainees with no more than two years of postdoctoral research experience are eligible for this award competition. The first-place winner for the Presidential Award of the Society will receive a plaque and a cash prize of \$600.00. The second-place winner will receive a plaque and a \$300.00 cash award. The winners are also eligible for a travel award if so requested at the time of application. Award recipients must be SLB members at the time the awards are presented. The candidates will present their papers at the Presidential Award Competition Session, date and time are to be announced.

2001 Awards Finalists

To be announced.

Travel Awards

Members of SLB who are predoctoral students or postdoctoral trainees, and are first presenting authors of their papers at the meeting are eligible for these travel awards.

2001 Recipients

To be announced.

This year's awards are sponsored by the Society for Leukocyte Biology.

The Cytokine Odyssey 2001

A joint meeting of the
INTERNATIONAL CYTOKINE SOCIETY
and the
SOCIETY FOR LEUKOCYTE BIOLOGY

Outrigger Wailea Resort, Maui, Hawaii

November 8-11, 2001

Program Committee: C. Ware (*Chair*), T. Hamilton, A. Mantovani
K. Matshushima, A. Miyajima, P. Murphy

PROGRAM

Thursday, November 8

- 8:30-3:00 PM **SLB COUNCIL MEETING**
(Renaissance Wailea Beach Resort,
Wailea Terrace)
- 12:00-7:00 PM **REGISTRATION**
(Aulani Lanai)
- 2:30-5:30 PM **ICS COUNCIL MEETING**
(Kukui Room)
- 5:00-6:00 PM **SLB PRESIDENTIAL STUDENTS
AWARDS IN RESEARCH**
(Jade/Plumeria/Maile)

Chair: S. Wahl
- 5:00 C. Rosenberger. Univ. of British Columbia.
See abstract 213, page 11.
- 5:15 T. Wang. Ohio State Univ.
See abstract 133, page 7.
- 5:30 F. Bäckhed. Karolinska Inst.
See abstract 207, page 11.
- 5:45 J. Lomas. Rhode Island Hosp.
See abstract 281, page 14.

6:30-8:10 PM **ICS PRESIDENTIAL ADDRESS, ICS
LIFETIME ACHIEVEMENT AWARDS
AND KEYNOTE ADDRESS**
(Jade/Plumeria/Maile)

Chair: J-M. Dayer

6:30 **PRESIDENTIAL ADDRESS. The saga of the
balance...or imbalance.** J-M. Dayer, ICS President, Univ. Hosp.,
Geneva.

1 6:50 **ICS LIFETIME AWARD. The suppressor of
cytokine signaling-1.** D. Metcalf. Walter and Eliza Hall Inst. of
Med. Res., Melbourne.

2 7:15 **ICS LIFETIME AWARD. Cytokine control of
hematopoiesis and leukemia: from basic biology to the clinic.**
L. Sachs. Weizmann Inst. of Sci., Rehovot.

7:40 **KEYNOTE ADDRESS. The dynamics of
primary and secondary T cell responses.** A. Lanzavecchia.
Basel Inst., Switzerland.

8:10-10:00 PM **WELCOME RECEPTION**
(Pacific Terrace)

Friday, November 9

7:00-8:30 AM **POSTER SESSION I SET-UP/CONTI-
NENTAL BREAKFAST**
(Haku/Pikake I & II)

8:00-5:00 PM **REGISTRATION**
(Aulani Lanai)

8:30-12:05 PM **PLENARY SESSION I: CYTOKINE SIGNALING PATHWAYS IN DEVELOPMENT AND EFFECTOR ACTION**
(Jade/Plumeria/Maile)

Cochairs: T. Hamilton and C. Ware

- 3 8:30 **Regulation of cytokine gene expression during T cell differentiation.** A. Rao, D.C. Solymar, D.U. Lee and S. Agarwal. Harvard Med. Sch. and Ctr. for Blood Res., Boston.
8:55 **TBA.** J. Allison*. Univ. of California, Berkeley.
- 4 9:20 **Semaphorins in the immune regulation.** A. Kumanogoh, K. Suzuki, C. Watanabe, S. Marukawa, N. Takegahara, E. Seng Ch'ng and H. Kikutani*. Osaka Univ.
9:45 **TCR signaling by NF AT in early T cell development.** G. Crabtree*. Stanford Univ.
10:05 **Break**
10:30 **NF- κ B: a key mediator of cytokine responses.** S. Ghosh. Yale Univ. Sch. of Med.
- 5 10:55 **Mechanisms of TRAF regulation in B lymphocyte signaling by CD40 and LMP1.** G.A. Bishop. Univ. of Iowa.
- 6 11:20 **Decoy receptors as a strategy to regulate inflammatory cytokines and chemokines.** A. Mantovani. Mario Negri Inst., Milan.
- 7 11:45 **Death domain-like modules in cell death and inflammation.** J. Tschopp. Univ. of Lausanne.
12:10 **Making T helper subsets.** R. Locksley*. UCSF/HHMI.
12:35 **Lunch (on own)**

1:30-5:05 PM **CONCURRENT SYMPOSIA 1-3**

1:30-5:05 PM **SYMPOSIUM 1: MECHANISMS OF SIGNAL TRANSDUCTION**
(Jade/Plumeria/Maile)

Cochairs: A. Rao and J. Tschopp

- 8 1:30 **The interaction between gp130 and viral interleukin-6: a paradigm for the architecture of gp130-cytokine signaling assemblies.** K.C. Garcia, X. He, S. Rose-John and D. Chow. Stanford Univ. Sch. of Med. and Christian Albrechts Univ. of Kiel, Germany.
- 9 1:50 **Genetic dissection of signaling in mice with targeted mutations in gp130.** B.J. Jenkins, C. Quilici, D. Grail, A. Giraud, A. Dunn and M. Ernst. Ludwig Inst. for Cancer Res., Melbourne.
- 10 2:05 **Analysis of lymphoid development and immune responses in rescued adult Jak1 deficient mice.** G. Schreiber, J.M. White and R.D. Schreiber. Washington Univ. Med. Sch.
- 11 2:20 **IFN β -induction of IL-1Ra synthesis in human monocytes involves PI 3-kinase-STAT1 signaling pathway.** N. Hyka, M-T. Kaufmann, D. Burger and J-M. Dayer. Univ. Hosp., Geneva.

- 12 **See Symposium 1, 4:20 PM.**
- 190 2:35 **Identification of a molecule critical for lymphocyte motility.** T. Sasazuki* and Y. Fukui. Kyushu Univ., Japan.
2:55 **Break**
- 13 3:30 **Molecular recognition between TRAF3 and TANK in downstream TNF signaling pathways.** K.R. Ely, C. Li, G. Cheng, J. He, A.C. Satterthwait and E. Cabezas. Burnham Inst., La Jolla and UCLA.
- 14 3:50 **Pathogenic signaling by flagellin via Toll-like receptor 5 activates the NF- κ B and the proinflammatory gene program.** J. DiDonato, A. Deb, T. Tallant and N. Kar. Cleveland Clin. Fndn.
- 15 4:05 **IKK α and IKK β have different roles in LT β R-mediated NF- κ B activation.** E. Dejardin, E. Haas, C. Ware and D. Green. La Jolla Inst. for Allergy and Immunol.
- 12 4:20 **Genetic analysis of NF- κ B-dependent signaling pathways.** X. Li. Cleveland Clin. Fndn.
- 16 4:35 **Cytokine-mediated activation of IKK is initiated in lipid rafts.** M. Delhase, D.M. Rothwarf and M. Karin. UCSD.
- 17 4:50 **ROS-dependent activation of interferon regulatory factor 3 by lipopolysaccharide.** O. Dang, L. Navarro and M. David. UCSD.

1:30-5:00 PM **SYMPOSIUM 2: REGULATION OF GENE EXPRESSION**

(South Pacific Ballroom)

Cochairs: D. Paulnock and S. Durum

- 18 1:30 **TNF- α gene regulation in LPS or mycobacteria-stimulated monocytes is dependent upon the assembly of a unique enhanceosome and CBP/p300.** A.E. Goldfeld, R. Barthel, A.V. Tsytsykova and A.K. Barczak. Ctr. for Blood Res., Harvard Med. Sch.
- 19 1:45 **IL-6 regulation of the human methyltransferase (HDNMT) gene in human erythroleukemia cells.** W.L. Farrar and D. Hodge. NCI-Frederick, SAIC Frederick, MD.
- 20 2:00 **Directed chromatin remodelling at cytokine gene promoters following T cell activation.** M.F. Shannon, S. Rao, A.F. Holloway, J. Attema, R. Reeves and S. Gerondakis. John Curtin Sch. of Med. Res., Australian Natl. Univ., Washington State Univ. and Walter and Eliza Hall Inst., Melbourne.
- 21 2:15 **Expression profiling in lymphotoxin and tumor necrosis factor knockout mice.** A.N. Shakhov and S.A. Nedospasov. SAIC Frederick, NCI-Frederick, MD and Engelhardt Inst. of Molec. Biol., Moscow.
- 22 2:30 **Transcriptional activation and repression: factor recruitment and GRIP1 corepressor activity at a collagenase-3 AP-1 element/tethering GRE.** I. Rogatsky, K.A. Zarembler and K.R. Yamamoto. UCSF.
- 23 2:45 **IL-7 controls chromatin accessibility for the TCR gamma V(D)J recombination via histone acetylation.** J. Huang, R. Murray, S.K. Durum and K. Muegge. NCI-Frederick Cancer R&D Ctr. and DNAX, Palo Alto.

* Member of US-Japan Immunology Board.

3:00 **Break**

24 3:30 **IL-1 differentially stabilizes ARE containing mRNAs.** J. Tebo, M.A. Frevel, B.R.G. Williams and T.A. Hamilton. Cleveland Clin. Fndn.

25 3:45 **Large-scale analysis of AU-rich element-containing mRNA expression and turnover using cDNA microarrays.** M.A. Frevel, T. Takheet, K. Stookey, K.S.A. Khabar and B.R.G. Williams. Cleveland Clin. Fndn. and King Faisal Spec. Hosp. and Res. Ctr., Riyadh, Saudi Arabia.

26 4:00 ***Mycobacterium avium* infection of macrophages induces protein binding to the 3'UTR of Nramp1 mRNA.** W.P. Lafuse, G.R. Alvarez and B.S. Zwilling. Ohio State Univ.

27 4:15 **Human IFN-gamma mRNA autoregulates its translation by strongly activating PKR.** R. Kaempfer, Y. Ben-Asouli, Y. Banai, Y. Pel-Or and A. Shir. Hebrew Univ.-Hadassah Med. Sch., Israel.

28 4:30 **Regulation of macrophage arginase I expression.** P.J. Murray, R. Rutschman, K. Baldovich, M. Hesse, T. Wynn and R. Lang. St. Jude Children's Res. Hosp. and NIAID, NIH.

29 4:45 **Colony stimulating factor-1 differentially regulates macrophage responses to CpG DNA and LPS.** M.J. Sweet, C.C. Campbell, D.P. Sester, D. Xu, K.J. Stacey, F.Y. Liew and D.A. Hume. Univ. of Queensland and Univ. of Glasgow.

1:30-5:00 PM **SYMPOSIUM 3: EMERGING CYTOKINES AND RECEPTORS**
(Puakenikeni I & II)

Chair: M. Feldman

30 1:30 **Null mutation of the TNF family member, APRIL, results in embryonic lethality and cardiac defects.** L. Runkel, F. Kuo, P. Rennert, Y. Chicheportiche, J. Tschopp and J.L. Browning. Biogen Inc., Cambridge, MA, Dana Farber Cancer Ctr., CMU, Geneva and Univ. of Lausanne.

31 1:45 **IL-22 is a tightly regulated IL-10-like molecule that induces an acute-phase response and renal tubular basophilia.** L.A. Fouser, A-J. Lambert, E. Clark, B. Deng, X-Y. Tan, V. Spaulding, I-M. Wang, M. Kobayashi M. Whitters, D. Thibodeaux, J. Leonard, V. Ling, P. Wu, B. Annis, Z. Lu, R. Zollner, K. Jacobs, B. Goad and D. Pittman. Genet. Inst., Cambridge, MA.

32 2:00 **Identification, cloning and characterization of a novel soluble receptor which binds IL-22 and neutralizes its activity.** S.V. Kotenko, L.S. Izotova, O.V. Mirochnitchenko, H.L. Dickensheets, R.P. Donnelly and S. Pestka. UMDNJ-New Jersey Med. Sch., UMDNJ-RW Johnson Med. Sch. and FDA, Bethesda.

33 2:15 **IL-18 binding protein protects against LPS-induced lethality and prevents the development of Fas/Fas ligand-mediated models of liver disease in mice.** R. Faggioni, R.C. Cattley, J. Guo, S. Flores, H. Brown, S. Scully, C. Chen, H. Yamane, T. Meng, F. Martin, S. Hu, T. Boone and G. Senaldi. Amgen Inc.

34 2:30 **Bax deletion restores thymocyte development in IL-7R α $^{-/-}$ mice.** S.K. Durum, K. Muegge and A.R. Khaled. NCI-Frederick, SAIC Frederick, MD.

35 2:45 **Coordination of interleukin-6 biology by soluble receptors.** S. Rose-John, M. Peters, T. Jostock, J. Müllberg, K.J. Kallen and J. Grötzinger. Univ. of Kiel, Germany.

3:00 **Break**

36 3:30 **Effects of IL-21 on murine T cells.** L.L. Carter, J. Jussif, L. Lowe, K. Johnson, M. Whitters, M. Kasaian, M. Collins, D. Young and B.M. Carreno. Genet. Inst./Wyeth Res., Cambridge, MA.

37 3:45 **Genomic characterization of light reveals linkage to an immune response locus on chromosome 19p13.3 and distinct isoforms generated by alternate splicing or proteolysis.** S.W. Granger, K.D. Butrovich, P. Houshmand, W.R. Edwards and C.F. Ware. La Jolla Inst. for Allergy and Immunol.

38 4:00 **Receptor-like properties of the 26 kDa trans-membrane form of TNF.** E. Duda, Á. Domonkos, A. Udvardy and L. László. Szeged Univ. and Eötvös Univ., Hungary.

39 4:15 **Pre-B cell enhancing factor inhibits apoptosis in inflammatory neutrophils.** S. Jia, J. Parodo, Y. Li, L. Fan and J.C. Marshall. Univ. Hlth. Network, Univ. of Toronto.

40 4:30 **Interleukin-1 homologue H4 is processed by caspase-1, binds to the IL-18 receptor but does not induce IFN- γ production.** S. Kumar, C. Hanning, M.R. Burke, D. Rieman, R. Lehr, F. Lynch and M. Lotze. GlaxoSmithKline Pharmaceut., King of Prussia and Qualtek Molec. Labs., Santa Barbara, CA.

41 4:45 **Combined cytokine stimulation of human naive CD4 $^{+}$ T cells results in IFN- γ production in absence of hypomethylation of the IFN- γ gene promoter.** F.W. Ruscetti, H.A. Young, C. Petrow-Sadowski, R. Bagni and J.A. Mikovits (Spon: C. Reynolds). NCI-Frederick, MD.

5:30-7:00 PM **POSTER SESSION I AND EXHIBITS**
(no host bar)

(Haku/Pikake I & II)

Poster board numbers are indicated in bold.

Chemokines and Chemokine Receptors

42 **1 Identification of the receptor that human LL-37 utilizes to activate human neutrophils, monocytes, and T cells.** D. Yang, Q. Chen, O. Chertov, M. Anderson, M. Hirata and J.J. Oppenheim. NCI, NIH, Frederick, MD, Magainin Res. Inst., Plymouth Meeting, PA and Iwate Med. Univ., Japan.

43 **2 CCR5-mediated Tyr phosphorylation is critical for RANTES-inducible T cell growth regulation and poxvirus infectivity.** E. Fish, M. Wong, J. Masters, A. Hinek, G. McFadden, S. Uddin and L. Plataniias. Univ. of Toronto, Univ. of Western Ontario and Univ. of Illinois at Chicago.

- 44 **3 Impaired hepatic granuloma formation in CCR2-deficient mice.** M. Takeya, K. Jinnouchi, Y. Terasaki, K. Takahashi, N. Maeda and W.A. Kuziel. Kumamoto Univ. Sch. of Med., Kumamoto, Japan, Univ. of Texas and Univ. of North Carolina Med. Sch.
- 45 **4 Herpes simplex virus selectively induce expression of the CC chemokine RANTES/CCL5 in macrophages through a mechanism dependent on PKR and ICP0.** J. Melchjorsen, F.S. Petersen, S.C. Mogensen and S.R. Paludan. Univ. of Aarhus, Denmark.
- 46 **5 CXC chemokine redundancy ensures local neutrophil recruitment during acute inflammation.** D.G. Remick, L.B. Green, D.E. Newcomb, S.J. Garg, G.L. Bolgos and D.R. Call. Univ. of Michigan.
- 47 **6 Selective chemokine down-regulation in U-937 PMA-primed cells by an anti-inflammatory oligopeptide produced by *Entamoeba histolytica*.** J.R. Velázquez, D. Utrera-Barillas, J.A. Enciso and R. Kretscher. IMSS, Mexico City.
- 48 **7 Structural and functional characterization of human CXCR4 as a chemokine receptor and HIV-1 co-receptor by mutagenesis and molecular modeling studies.** N. Zhou, Z. Luo, J. Luo, D. Liu, J.W. Hall, R.J. Pomerantz and Z. Huang. Jefferson Med. Col. and Sch. of Molec. and Cell. Biol., Univ. of Illinois at Urbana-Champaign.
- 49 **8 CXCL10 inhibits CXCL1-induced signaling by interrupting CXCL1 binding activity to CXCR2 receptor.** D. Wang and A. Richmond. DVA Med. Ctr., Nashville and Vanderbilt Univ. Sch. of Med.
- 50 **9 Expression of growth-related oncogene- α by human eosinophilic granulocytes.** T. Persson, P. Andersson, A. Bjartell and A. Egesten. Lund Univ. and Univ. Hosp., Malmö, Sweden.
- 51 **10 Detection of a novel component of CCR5 ligand induced trafficking.** O.M.Z. Howard, B. Gertz, J. Wooters, S. Lockett and J.J. Oppenheim. SAIC Frederick, NCI-Frederick, MD and Genet. Inst., Cambridge, MA.
- 52 **11 Truncation of N-terminal amino acid residues of leukotactin-1 increases agonistic potency on CCR1 and CCR3.** C-K. Lee, J.K. Lee, H.S. Kim, K. Kim, K. Kwack, D.S. Na and B.S. Kwon. Col. of Pharm., Chungbuk Natl. Univ., Sahm-Yook Univ. and Univ. of Ulsan, Republic of Korea.
- 53 **12 Identification of cis-acting DNA elements required for PMA-induced transcription of the human leukotactin-1 and Ck β 8 genes.** Y. Shin, I. Song and J. Kim. Sch. of Life Sci. and Biotechnol., Kyung Hee Univ., Republic of Korea.
- 54 **13 Leptin induces the secretion of IP-10, but not RANTES, on human monocytic cells.** J-M. Dayer, R. Chicheportiche and C.A. Meier. Univ. Hosp., Geneva.
- 55 **14 Macrophage inflammatory protein-3 α expression is increased in human inflammatory bowel disease.** A. Kaser, O. Ludwiczek, F.A. Offner and H. Tilg. Univ. Hosp. Innsbruck and Academic Teaching Hosp. Feldkirch, Austria.

Proinflammatory Signaling Pathways

- 56 **15 Differential regulation of IP-10 expression in human airway smooth muscle by INF γ and TNF- α : role of protein kinase C.** E. Hardaker, M. Comegys, H. Sarau and K. Belmonte. GlaxoSmithKline, Resp., King of Prussia.
- 57 **16 Regulation of IL-8 production after human keratinocytes UVB-irradiation.** A. Grandjean-Laquerriere, S.C. Gangloff, R. Le Naour, C. Trentesaux, W. Hornebeck and M. Guenounou. INSERM, CNRS, Reims.
- 58 **17 TNF receptor associated factor (TRAF) 1 is a negative regulator of TNFR2 signaling: enhanced TNF signaling in TRAF 1-deficient mice.** E.N. Tsitsikov, D. Laouni, I.F. Dunn, T.N. Sannikova, L. Davidson, F.W. Alt and R.S. Geha. Children's Hosp. and Harvard Med. Sch.
- 59 **18 Direct effect of proinflammatory cytokines on human myocardial contractile function if ICE dependent and is mediated by NO.** B.J. Pomerantz, L.L. Reznikov, A.H. Harken and C.A. Dinarello. Univ. of Colorado Hlth. Sci. Ctr.
- 60 **19 Differential regulation of Toll-like receptor mRNAs by microbes, their products and cytokines.** K. Zarembek, D. Monack, S. Falkow and P. Godowski. Genentech and Stanford Univ.
- 61 **20 Injury augments Toll-like receptor responses independently of TLR4.** J.A. Lederer, E.M. Purcell, O.P. Shelley, J.A. Mannick and H.M. Paterson. Brigham and Women's Hosp., Harvard Med. Sch.
- 62 **21 Mal (MyD88 adapter-like) is required for Toll-like receptor-4 signal transduction.** K.A. Fitzgerald, E.M. Palsson-McDermott, A.G. Bowie, C.A. Jeffries, A. Mansell, G. Brady, B. Brint, A. Dunne, P. Gray, M.T. Harte, D. McMurray, D.E. Smith, J.E. Sims, T.A. Bird and L.A.J. O'Neill. Trinity Col. Dublin and Immunex Corp., Seattle.

NF-Kappa B

- 63 **22 Prion protein activation of the NF- κ B signalling pathway in human monocyte-derived dendritic cells.** S.M. Bacot, M. Jessen and G.M. Feldman. FDA, Bethesda.
- 64 **23 Both IKK α and IKK β are required to phosphorylate and activate the p65 subunit of NF- κ B in response to cytokine-stimulated phosphatidylinositol-3-kinase and Akt.** N. Sizemore, N. Lerner, N. Dombrowski and G.R. Stark. Cleveland Clin. Fndn.
- 65 **24 The synergistic effect of IL-1 β on induction of C-reactive protein by IL-6 can be reproduced by overexpressed NF- κ B.** A. Agrawal, H. Cha-Molstad, D. Samols and I. Kushner. Case Western Reserve Univ. and MetroHlth. Med. Ctr., Cleveland.

MAPK, Stress Pathway

- 66 **25 An MEK inhibitor, PD98059 enhances IL-1-induced NF- κ B activation by the enhance and sustained degradation of I κ B α .** T. Kasahara, M. Funakoshi, Y. Sonoda and K. Tago. Kyoritsu Col. of Pharm. and Jichi Med. Sch., Japan.

- 67 **26 Involvement of p38 in the regulation of inflammatory and Th1/Th2 cytokines.** S. Gupta, S. Crouse, J. Chou, B. Shuman and K. Reagan. BioSource Intl. Inc., Camarillo, CA.
- 68 **27 LPS signaled ERK activity is augmented by substance P in human PMN.** J.C. Friel, R.B. Zurier, C. Miller-Graziano and P.E. Bankey. Univ. of Massachusetts Med. Sch. and Univ. of Rochester.

JAK/STAT

- 69 **28 Definition of Tyk2-binding domains in platelet-activating factor receptor.** V. Lukashova, Z. Chen, M. Rola-Pleszczynski and J. Stankova. Univ. of Sherbrooke, Canada.
- 70 **29 JAB/SOCS1/SSI-1 is an IL-2-induced inhibitor of IL-2 signaling.** B. Sporri, P.E. Kovanen, A. Sasaki, A. Yoshimura and W.J. Leonard. NHLBI, NIH.

SMADs

- 71 **30 Expression of inhibitory Smad7 in experimental uveitis.** J.B. Allen and K.M. Pittman. North Carolina State Univ. Col. of Vet. Med.

Novel Cytokines and Cytokine Function

- 72 **31 Distinct signal transduction pathways induced by IL-2 and IL-2/15R β agonists.** R. Eckenberg, T. Rose, J.-L. Moreau, R. Weil, F. Gesbert, S. Dubois, D. Tello, M. Bossus, H. Gras, A. Tartar, J. Bertoglio, S. Chouaib, M. Goldberg, Y. Jacques, P.M. Alzari and J. Thèze. Pasteur Inst. and INSERM.
- 73 **32 Expression of alternative spliced variants of cytokine mRNA in human erythroid cells.** S.V. Sennikov, A.N. Silkov, S.V. Krysov, T.V. Injelevskaya and V.A. Kozlov. Inst. of Clin. immunol. SB RAMS, Novosibirsk, Russia.
- 74 **33 IL-1H4 (IL-1F7), a novel member of the interleukin-1 gene family, is regulated by LPS and induces IL-8 but not IFN- γ .** P. Bufler, S.-H. Kim, S. Kumar and C.A. Dinarello. Univ. of Colorado Hlth. Sci. Ctr. and GlaxoSmithKline, King of Prussia.
- 75 **34 IL-21 receptor expression and modulation of gene transcription by interleukin 21.** M. Whitters, J. Calvetti, K. Johnson, J. Witek, M. Byrne, L. Lowe, B. Sibley, M. Collins and D. Young. Genet. Inst./Wyeth-Ayerst Res., Cambridge, MA.
- 76 **35 IL-21 blocks IL-15-induced NK cell expansion and enhances IFN- γ production.** M. Kasaian, M. Whitters, K. Johnson, R. Konz, B. Deng, L.L. Carter, M. Collins and D. Young. Genet. Inst./Wyeth Res., Cambridge, MA.
- 77 **36 Hyper-6: a new designer cytokine-modified melanoma vaccine: clinical results of a phase II study.** A. Mackiewicz, S. Nawrocki and S. Rose-John. Great Poland Cancer Ctr., Poznan.

Interferons and IFN Receptors

- 78 **37 PEG Intron and interferon alpha-2b are comparable in regulating gene-expression for relevant anti-viral immuno-responsiveness.** R. Bordens, D. Brassard, M. DeLorenzo and M. Grace. Schering-Plough Res. Inst., Union, NJ.
- 79 **38 Interferon signaling requires specific phosphotyrosines located within the intracellular domain of IFNAR2c.** T.C. Wagner, S. Velichko, D. Vogel, M.R.S. Rani, S. Leung, O. Colamonici, R.M. Ransohoff, H.D. Perez and E. Croze. Berlex Biosci., Richmond, CA, Cleveland Clin. Fdn. and Univ. of Illinois at Chicago.
- 80 **39 A comparison of the anti-viral efficacy of murine type I IFN transgenes against herpes simplex virus type 1.** D.J.J. Carr, P. Haerle, M.-P. AgBaga, V. Cull and C. Lawson. Univ. of Oklahoma Hlth. Sci. Ctr. and Murdoch Univ., Australia.
- 81 **40 Withdrawn.**

- 82 **41 Characterization of pathways promoting IFN- α/β expression during *in vivo* viral infections.** L. Malmgaard, M. Dalod, T.P. Salazar-Mather, C. Lewis and C.A. Biron. Univ. of Aarhus, Denmark and Brown Univ.
- 83 **42 The 2',5'-oligoadenylate synthetase gene in mice.** S. Kakuta, S. Shibata and Y. Iwakura. Univ. of Tokyo.

Neutrophil Activation

- 84 **43 Oxidative burst by Toll-like receptors and CD14 in avian heterophils stimulated with bacterial components.** M.B. Farnell, T.C. Crippen, H. He and M.H. Kogut. Col. of Vet. Med., Texas A&M Univ. and USDA, College Station, TX.
- 85 **44 Activation of chicken immune cells by unmethylated CPG dinucleotide motif of bacterial DNA.** H. He, M.B. Farnell, T.L. Crippen, M.H. Kogut. USDA, College Station, TX.
- 86 **45 Preservation of the pattern of tyrosine phosphorylation in human neutrophil lysates. II. A sequential lysis protocol for the analysis of tyrosine phosphorylation-dependent signaling.** C. Gilbert, E. Rollet-Labelle and P.H. Naccache. CHUL and Fac. of Med., Laval Univ., Canada.
- 87 **46 Diverging effects of γ interferon and TNF- α on polymorphonuclear neutrophils: induction of cytokines synthesis, receptor expression and differentiation to antigen-presenting cells.** G.M. Hänsch, C. Iking-Konert and C. Wagner. Univ. of Heidelberg.
- 88 **47 Expression of granzyme B and perforin in polymorphonuclear neutrophils: identification and functional analysis.** C. Wagner, C. Iking-Konert and G.M. Hänsch. Univ. of Heidelberg.
- 89 **48 Stealth LPS from *Prevotella sp.*** K.M. Pabst and M.J. Pabst. Univ. of Tennessee, Memphis.

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- 91 **50** **Inhibitory actions of glucosamine, a therapeutic agent for osteoarthritis, on the functions of neutrophils.** I. Nagaoka, J. Hua, K. Iwabuchi, Y. Tsutsumi-Ishii, F. Niyonsaba and K. Sakamoto. Juntendo Univ. Sch. of Med., Japan and Koyo Chem. Co. Ltd., Tokyo.
- 92 **51** **Transferrin-derived phagocytosis activators (MAPPs): characterization of the transferrin microheterogeneity.** H. Sakamoto, M. Ueno, W. Yanghong, R. Khatun, S. Tanaka, M. Onodera and K. Miyabe. Kagawa Med. Univ., Japan.

Macrophage Activation

- 93 **52** **Regulation of cyclooxygenase (COX)-2 expression in macrophages: signaling pathways of the RON receptor tyrosine kinase that inhibit LPS-induced COX-2 gene transcription.** M-H. Wang, D. Wang, Y-Q. Zhou and Y-Q. Chen. Univ. of Colorado Hlth. Sci. Ctr. and Denver Hlth. Med. Ctr. and First Affiliated Hosp., Zhejiang Univ. Sch. of Med., China.
- 94 **53** **Expression of cytokine-related genes in leukocytes infected with herpes simplex virus 1 and 2: comparison between resistant and susceptible mice strains.** S.R. Paludan and S.C. Mogensen. Univ. of Aarhus, Denmark.
- 95 **54** **PMA inhibits plasmin-mediated release of aggregated LDL from macrophages.** H.S. Kruth, W. Huang, W-Y. Zhang and I. Ishii. NHLBI, NIH.
- 96 **55** **Serotonin modulates the production of mediators by alveolar macrophages.** E. Bissonnette and G. Ménard (Spon: J. Stankova). Hosp. Ctr., Univ. of Laval, Canada.
- 97 **56** **Multiple distinct signal transduction pathways are induced by Toll-like receptors 2 and 4.** T.K. Means, B.W. Jones, K.A. Heldwein and M.J. Fenton. Boston Univ. Sch. of Med.
- 98 **57** **Roles of Toll-like receptors in immunity against mycobacteria.** K.A. Heldwein, T.K. Andresen, T.K. Means, S.N. Vogel and M.J. Fenton. Boston Univ. Sch. of Med. and Uniformed Serv. Univ. of Hlth. Sci.
- 99 **58** **Proteome of monocytes primed by LPS.** M.J. Pabst. Univ. of Tennessee, Memphis.
- 100 **59** **Demonstration of a macrophage proliferation inhibition factor from human decidua distinct from transforming growth factor beta.** L-J. Eales-Reynolds, Y. Dang, T. Rustam and P. Naylor. Sch. of Biomed. and Life Sci., Univ. of Surrey.
- 101 **60** **Murine macrophages infected with *Leishmania* major exhibit reduced nitric oxide production associated with alterations in potassium channel activity.** K. Scott, J.L. Stafford, F. Galvez, G.G. Goss and M. Belosevic. Univ. of Alberta.

- 102 **61** **DAP12: possible role in Fc γ R-mediated phagocytosis.** A. Longtin, A. Descoteaux and P. Duplay. INRS-Inst. Armand-Frappier, Laval, Canada.
- 103 **62** **Unregulated signaling pathways in TGF- β 1-deficient mice.** N.L. McCartney-Francis and S.M. Wahl. NIDCR, NIH.
- 104 **63** **The structure of LPS and lipid A influences the activation of murine macrophages via CD14-dependent or CD14-independent pathways.** S.C. Gangloff, U. Zähringer, C. Blondin and S.M. Goyert. Res. Ctr. Borstel, Germany and North Shore Univ. Hosp./NYU Sch. of Med.
- 105 **64** **Both platycodin D and D3 isolated from the root of *Platycodon grandiflorum* inhibit production of nitric oxide in activated RAW 264.7 cells but not TNF- α secretion.** G. Schuller-Levis, E.B. Lee, C. Wang, W. Levis, D.W. Lee and E. Park. New York State Inst. for Basic Res. in Develop. Disabil., Staten Island and Seoul Natl. Univ.

Lymphocyte Activation

- 106 **65** **The novel, MHC class-I related molecules, ULPB1 and ULPB2, bind to the NKG2D/DAP10 complex, resulting in functional activation of both human and murine NK cells.** J. Chalupny, P.V. Sivakumar, C.L. Sutherland, J. Mullberg, W. Chin, G. Jackson, M. Kubin and D. Cosman. Immunex Corp., Seattle.
- 107 **66** **Lymphocyte-platelet adhesion in IL 2 therapy of patients with laryngeal carcinoma.** Y. Vitkovsky, L. Ilynykh, A. Solpov and B. Kuznik. Chita Med. Acad., Russia.
- 108 **67** **Massive activation of B cells in mice following a primary rotavirus infection.** S.E. Blutt, K.L. Warfield, D.E. Lewis and M.E. Conner. Baylor Col. of Med.

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- 109 **68** **Identification of alpha-defensin-1 (HNP-1) as an anti-chemotactic agent for human polymorphonuclear leukocytes.** P.S. Grutkoski, C.T. Graeber, A. Ayala and H.H. Simms. Rhode Island Hosp., Providence and NYU Sch. of Med., LIJ-North Shore Hosps.
- 110 **69** **Identification of a modified form of the vitamin D binding protein that functions as a co-chemotactic factor for C5a.** G. Trujillo and R.R. Kew. SUNY at Stony Brook.

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- 111 **70** **Inhibition of the chemiluminescence of human polymorphonuclear leukocytes and carrageenan-induced rat's paw oedema by a newly synthesized 4-amino uracil derivative.** A.A. Mustafa, A.S. Al-Tuwaijri, M.S. Al-Humayyd and L.Y. Al-Ayadhi. King Saud Univ. Col. of Med., Saudi Arabia.
- 112 **71** **Macrophage migration inhibitory factor (MIF) interacts with the vesicle-tethering protein p115: a possible route for the secretion of leaderless MIF.** J. Baugh, X. Lin, R. Mitchell and R. Bucala. Picower Inst. for Med. Res., Manhasset, NY.

NADPH Oxidase

- 113 72 **Cloning of the rabbit leukocyte NADPH oxidase.** K.A. Gauss, P.L. Mascolo, D.W. Siemsen, L.K. Nelson, P.L. Bunger, P.J. Pagano and M.T. Quinn. Montana State Univ. and Henry Ford Hosp., Detroit.
- 114 73 **Regulation of the NADPH oxidase of neutrophils by Rac2 and RhoGDI.** B. Diebold and G. Bokoch. Scripps Res. Inst.

Effector Mechanisms of Phagocytes

- 115 74 **Differential nitric oxide production by immune cells of chickens.** T.L. Crippen, L.H. Ripley, M.B. Farnell, V.K. Lowry, C.L. Sheffield and M.H. Kogut. USDA, College Station, TX and Col. of Vet. Med., Texas A&M Univ.
- 116 75 **The consensus transport signature sequence of Nramp1 is required for iron transport.** C.M. Bishop, D.E. Kuhn, W.P. Lafuse and B.S. Zwilling. Ohio State Univ.
- 117 76 **Increased resistance to mycobacterial growth by alpha-2 adrenergic stimulation of macrophages requires both nitric oxide and superoxide.** K.M. Weatherby, W.P. Lafuse and B.S. Zwilling. Ohio State Univ.
- 118 77 **Role for bystander cell participation and cognate cytokine production for rapid induction of human CD8⁺ T cell effector function.** G.T. Brice, N.L. Graber, D.J. Carucci and D.L. Doolan. Naval Med. Res. Ctr., Silver Spring, MD.

Cytokines Superfamily Members

- 119 78 **Generation of antagonists by amino acid replacement in the D-helix of human IL-21.** C. Brandt, C. Birks, C. Chan, H. Liu, C. Ostrander, T. Pownder, P. McKernan, D. Foster and J. West. ZymoGenetics Inc., Seattle.
- 120 79 **Cytokine-synthesizing activity of erythroid nuclear cells of human bone marrow.** V.A. Kozlov, S.V. Sennikov, T.V. Injelevskaya, I.B. Kovinev and M.I. Loseva. Inst. of Clin. Immunol. SB RAMS, Novosibirsk Med. Acad., Russia.
- 121 80 **Presence of a nuclear export signal-like sequence within the structure of the human interleukin-1 α precursor.** M. Sudo, N. Watanabe and Y. Kobayashi. Toho Univ., Japan.
- 122 81 **Interleukin 18 is involved in acetaminophen-induced liver injury in a Fas/Fas ligand independent mechanism.** J.C. Waksman, G.B. Bogdan, R.C. Dart, C.A. Dinarello and G. Fantuzzi. Denver Hlth., Univ. of Colorado Hlth. Sci. Ctr.

TNF-Related Cytokines and Receptors

- 123 82 **Inhibition of experimental autoimmune encephalomyelitis by an antibody specific for mouse CD30L.** M. Kennedy, P.V. Sivakumar, S. Brown, C. Smith, K. Majeskey, N. Boiani, R. Goodwin and K. Mohler. Immunex Corp., Seattle.

- 124 83 **Lymphotoxin (LT) function *in vivo* as dissected by inactivation of LT complex in B cells.** A.V. Tumanov, D.V. Kuprash, M.A. Lagarkova, K. Abe, A.N. Shakhov, C. Stewart, A.V. Chervonsky and S.A. Nedospasov. Engelhardt Inst. of Molec. Biol., Moscow, SAIC Frederick, NCI-Frederick, MD and The Jackson Lab., Bar Harbor, ME.
- 125 84 **Dual function of TNF as regulator of myelopoiesis in long-term bone marrow cultures.** M. Drutska, D.V. Kuprash, S.A. Nedospasov and J. Keller. Engelhardt Inst. of Molec. Biol., Moscow and SAIC Frederick NCI-Frederick, MD.
- 126 85 **Biological functions of tumor necrosis factor and lymphotoxin *in vivo* assessed using a novel panel of knockout mice.** S.A. Nedospasov. SAIC Frederick, NCI Frederick, MD and Engelhardt Inst. of Molec. Biol., Moscow.
- 127 86 **Modulation of death receptors by adenovirus E3 proteins requires intracellular-trafficking motifs.** P.S. Norris, V. Piguet, C.A. Benedict, K.D. Butrovich and C.F. Ware. La Jolla Inst. for Allergy and Immunol. and Univ. Hosp. of Geneva.

Genomics of Cytokine Gene Expression

- 128 87 **Generation of diversity in the innate immune system: macrophage heterogeneity arises from gene-autonomous transcriptional probability of individual inducible genes.** T. Ravasi, C. Wells, A. Forest, D.M. Underhill, B.J. Wainwright, A. Aderem, S. Grimmond and D.A. Hume. Univ. of Queensland, Australia and Inst. for Systems Biol., Seattle.

Genetics of Cytokine Genes/Disease Linkage

- 129 88 **Interleukin-1 complex genotype is associated with susceptibility to asthma but only in males.** M. Hurme, M.M. Nieminen, A. Aromaa, T. Klaukka and J. Karjalainen. Univ. of Tampere Med. Sch. and Tampere Univ. Hosp., Finland.
- 130 89 **Contrasting evolution of the human leukocyte N-formylpeptide receptor subtypes FPR and FPRL1R.** A. Sahagun-Ruiz, J.S. Colla, J. Juhn, J.-L. Gao, P.M. Murphy and D.H. McDermott. NIAID, NIH.

Cytokine Gene Regulation

- 131 90 **Pleiotropic effects of IL-2 at the molecular level.** J. Reddy, P. Chastagner, S. Herblot, L. Fiette, L. Samady, X. Liu, J. Bonnet and J. Thèze. Pasteur Inst. and Shanghai Inst. of Biochem., China.
- 132 91 **The G-174 polymorphism of IL-6 alters binding of a HeLa transcription factor and forms functionally significant allelic associations with other 5'-flanking region polymorphisms.** R. Jeffery, L. Luong, C. Xia, E. Ogilvie, S. Humphries and P. Woo. University Col. London.
- 133 92 **NF- κ B-mediated TLR2 gene expression requires the transcription factor Sp1.** T. Wang, W.P. Lafuse and B.S. Zwilling. Ohio State Univ.

- 134 **93 Accelerated wound healing in TNF receptor p55 deficient mice with reduced leukocyte infiltration.** R. Mori, T. Kondo, Y. Ishida, N. Mukaida and T. Ohshima. Kanazawa Univ., Japan.
- 135 **94 IFN- α and IL-18 synergistically enhance IFN- γ production in human NK cells: differential regulation of Stat4 activation and IFN- γ gene expression by IFN- α and IL-12.** S. Matikainen, A. Paananen, M. Miettinen, A. Lehtonen, M. Kurimoto, T. Timonen, I. Julkunen and T. Sareneva. Natl. Publ. Hlth. Inst., Helsinki.
- 136 **95 Direct cytokine mRNA quantitation with the Invader[®] Assay.** T.Y. Takova, M.C. Olson, P.S. Eis, K.L. Vedvik, B.T. Argue, S.M. Olson, M.L. Curtis, L.M. Chehak, L.C. Mueller, R.W. Kwiatkowski. Third Wave Technol. Inc., Madison, WI.
- 137 **96 Inducer-specific enhanceosome formation controls TNF- α gene expression in T lymphocytes.** A.V. Tsytyskova, R. Barthel and A.E. Goldfeld. Ctr. for Blood Res., Harvard Med. Sch.
- 138 **97 MCMV infection downregulates cytokine gene expression in microvascular endothelial cells.** D. Hamamdziec and E.C. LeRoy. Med. Univ. of South Carolina.
- 139 **98 hBD-2 gene regulation in epithelial cells by macrophage-derived pro-inflammatory cytokines.** Y. Tsutsumi-Ishii, F. Niyonsaba and I. Nagaoka. Juntendo Univ. Sch. of Med., Japan.
- 140 **99 Identification of transcriptional domains of human lactoferrin and its roles in transcription of human IL-1 β gene in mammalian cells.** K-N. Son, C-K. Chung, J. Park and J. Kim. Sch. of Life Sci. and Biotechnol., Kyung Hee Univ., Republic of Korea.
- 141 **100 TGF β selectively regulates LPS-induced chemokine gene transcription.** Y. Dai, M. Novotny and T.A. Hamilton. Cleveland Clin. Fndn.
- 146 **105 Modulation of leukotriene B4 receptor-1 expression by dexamethasone: potential mechanism for enhanced neutrophil survival.** M. Rola-Pleszczynski, S. Turcotte and J. Stankova. Fac. of Med., Univ. of Sherbrooke, Canada.
- 147 **106 Potentiation by human serum of anti-inflammatory cytokine production by human macrophages in response to apoptotic cells.** K. Kurosaka, N. Watanabe and Y. Kobayashi. Toho Univ., Japan.
- 148 **107 TGF- β 1 is a target molecule in bisphosphonate-induced apoptosis in human neoplastic cells.** M. Shehata, J.D. Schwarzeimer, R. Hubmann and M. Hilgarth. Univ. of Vienna.
- 149 **108 Sayrin-1 is an antiapoptotic factor that blocks TNF and Fas-mediated cell death.** N. Pratt, J. Heath, E. Choi, Y. Zhao and N-S. Chang. Guthrie Res. Inst., Sayre, PA.
- 150 **109 TNF sensitizes Fas-mediated apoptosis irrespective of its anti-apoptotic effects in follicular dendritic cells.** S-M. Park and T.H. Lee. Yonsei Univ., Republic of Korea.
- 151 **110 A molecular basis for the endotoxin resistance of MIF-knockout mice: MIF inhibits cellular p53 accumulation via Cox-2 induction.** R.A. Mitchell, H. Liao, J. Chesney, J.A. Baugh, G. Fingerle-Rowson, J. David and R. Bucala. Picower Inst. for Med. Res., New York and Harvard Sch. of Publ. Hlth.
- 152 **111 Regulation of apoptotic cell death by interleukin-6 and oncostatin M.** S. Smola-Hess and U. Sandaradura de Silva. Univ. of Cologne.
- 153 **112 Characterization of a T-cell receptor signaling-deficient Jurkat cell variant that exhibits type 1 interferon-induced apoptosis.** A.M. Gamero, R. Abraham and A. Lerner. Cleveland Clin. Fndn.

Cytokine and Chemotactin Receptors

- 154 **113 Serum soluble macrophage colony-stimulating factor receptor level in patients with disease of cardiovascular system and kidneys.** F-Y. Bi, Q. Rao and K-F. Wu. Inst. of Hematol., Chinese Acad. of Med. Sci., Tianjin.
- 155 **114 Inflammatory stimuli regulate the secretion of the soluble human GM-CSF receptor by monocytes and granulocytes.** J.M. Prevost, J.L. Pelley, G.E. D'Egidio, P.P. Beaudry, C. Pihl, W. Zhu, E. Claret, J. Wijdenes and C.B. Brown. Univ. of Calgary, Canada.
- 156 **115 The role of galectin-3 in macrophage spreading.** E.P. Mayer, R. Nagaic, A. Ghaffar and M. Nachtigal. Univ. of South Carolina Sch. of Med. and Kumamoto Univ. Sch. of Med., Japan.

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- 157 **116 The rescue of SSI-1/SOCS-1 KO mice by lymphoid-specific restoration of SSI-1/SOCS-1 expression.** M. Fujimoto, T. Naka, H. Tsutsui, E. Seki, M. Himeno, T. Abe, A. Kimura, K. Nakanishi and T. Kishimoto. Osaka Univ. and Immunol. and Med. Zool., Hyogo, Japan.

Posttranslational Regulation

- 142 **101 Unstimulated human CD4 lymphocytes express a cytoplasmic immature form of the common cytokine receptor G chain.** L. Bani, V. Pasquier, M. Kryworuchko, J. Salamero and J. Thèze. Pasteur Inst.
- 143 **102 Stimulus-coupled IL-1 β posttranslational processing: identification of a novel class of inhibitors.** R. Laliberte, D. Perregaux, J. Eggler, M. Dombroski, R. Griffiths and C.A. Gabel. Pfizer Inc.

Apoptosis

- 144 **103 TIAF1 is an effector of transforming growth factor- β 1-mediated growth suppression and promotion.** N-S. Chang, S. Khera, L. Schultz, D. Sleve, J. Heath and R. Ruiz-Velasco. Guthrie Res. Inst., Sayre, PA.
- 145 **104 The non-ankyrin C-terminus of I κ B α physically interacts with p53 *in vivo* and inhibits transforming growth factor- β 1-mediated growth suppression.** J. Doherty, L. Schultz, J. Heath and N-S. Chang. Guthrie Res. Inst., Sayre, PA.

158 117 **Self-deactivating macrophages: transgenic overexpression of IL-10 directed by the CD68 promoter impairs inflammatory macrophage responses.** R. Lang, R.L. Rutschman, D. Greaves and P.J. Murray. St. Jude Children's Res. Hosp.

Receptor-Ligand Interactions

159 118 **Cognate interactions mediated by a recombinant LFA-3/IgG1 fusion induce CD16 signaling and granzyme B-dependent apoptosis of activated CD2⁺ cells.** A.J. da Silva, M. Brickelmaier, G. Majeau, Z. Li and P. Hochman. Biogen Inc, Cambridge, MA.

160 119 **IL-18 mutants with enhanced biological activities and decreased IL-18BP neutralization.** S-H. Kim, T. Azam, D-Y. Yoon, D. Novick, M. Rubinstein, G. Senaldi and C.A. Dinarello. Univ. of Colorado Hlth. Sci. Ctr.

Signal Transduction Pathways

161 120 **Functional dissection of the interleukin-1 receptor associated kinase domains.** L. Li, J. Hu and C. McCall. Wake Forest Univ. Sch. of Med.

162 121 **Detection of protein tyrosine kinase in immature chicken heterophils.** V.K. Lowry, M.B. Farnell, C.L. Swaggerty and M.H. Kogut. Texas A&M Univ. and USDA, College Station, TX.

163 122 **Design and application of a cytokine receptor-based interaction trap.** J. Tavernier, S. Eyckerman, I. Lemmens, J. Vandekerckhove, J. Van Der Heyden, X. Van Ostade, A. Verhee and L. Zabeau. Ghent Univ., Belgium.

164 123 **TLR2 and TLR4 ligands differentially regulate IL-1Ra gene expression in macrophages.** M.F. Smith, K. Brown-Steinke and V.S. Carl. Univ. of Virginia.

165 124 **Regulation of TNF- α and caspases by IFN- γ results in differential MMP-1 and MMP-9 production by GM-CSF and TNF- α -treated monocytes.** M. Zhou, Y. Zhang, J.A. Ardans and L.M. Wahl. NIDCR, NIH.

166 125 **The C-terminal part of IL-10 is regulating proliferation and apoptosis in hepato-cellular carcinoma cells through a p53 mechanism.** B. Gesser and C.G. Larsen. Univ. of Aarhus, Denmark.

167 126 **The roles of Rho GTPases in chemotaxis and chemokine receptor internalization.** J. Sai and A. Richmond. Vanderbilt Univ.

168 127 **Phosphorylation site-specific immunoassays for quantitation of the phosphorylation and activation of human epidermal growth factor.** J. Wang, D. Brumm, A. Colbert, E. Schaefer, L. Zorn and K. Reagan. BioSource Intl. Inc., Camarillo, CA.

169 128 **IRAK functions as a scaffolding protein in IL-1 signaling pathway.** Z. Jiang and X. Li. Cleveland Clin. Fndn.

170 129 **The role of M-Ras in the action and production of cytokines.** J. Schrader, A. Schallhorn, X. Guo, B. Wang and G. Ehrhardt. Univ. of British Columbia.

171 130 **The mitogen-activated protein kinase MKK6 physically interacts with the double-stranded RNA-dependent protein kinase PKR.** A.M. Silva, Z. Xu and B.R.G. Williams. Cleveland Clin. Fndn.

172 131 **Cytosolic phospholipase A2 is essential for prostaglandins but not for leukotrienes formation in phagocytic-like cells.** R. Levy, I. Liberty, I. Pesach, N. Haddad, L. Richel, R. Dana and F. Schlaeffer. Ben-Gurion Univ. of the Negev and Soroka Med. Ctr., Beersheba, Israel.

173 132 **T1/ST2 signal transduction: activation of AP-1, JNK, p38 and p42/p44 MAP kinase but not NF- κ B.** B. Brint, K.A. Fitzgerald and L.A.J. O'Neill. Trinity Col. Dublin.

174 133 **Suppressor of cytokine signaling (SOCS)3: role in suppression of macrophage activation by LPS.** F. Bazzoni, C. Berlatto, L. Gatto and M.A. Cassatella. Univ. of Verona.

8:00-10:00 PM **POSTER DISCUSSIONS/WORKSHOPS**

Posters will be selected for discussion/workshops.

Saturday, November 10

7:00-8:30 AM **POSTER SESSION II SET-UP/CONTINENTAL BREAKFAST**
(Haku/Pikake I & II)

8:00-5:00 PM **REGISTRATION**
(Aulani Lanai)

8:30-10:10 AM **PLENARY SESSION II: TRANSITIONS IN INNATE AND ADAPTIVE IMMUNITY**
(Jade/Plumeria/Maile)

Chair: L.L. Lanier

175 8:30 **Stress management by NK cells: NKG2D receptor recognition of MHC class I-like antigens on tumors and virus-infected cells.** L.L. Lanier. UCSF.

176 8:55 **The immunological synapse: coordinating T cell migration and antigen recognition.** M.L. Dustin. NYU Med. Ctr.

177 9:20 **The role of FADD in cytokine-mediated T cell activation.** C.M. Walsh, D. Beisner, I. Catlett and S.M. Hedrick*. UCSD.

9:45 **Regulation of class switching by cytokines.** T. Honjo*. Kyoto Univ.

10:10 **Break**

* Member of US-Japan Immunology Board.

10:40-12:20 PM PLENARY SESSION III: INFECTIOUS DISEASES

(Jade/Plumeria/Maile)

*Chair: C. Biron***10:40 Balancing innate IFN-alpha/beta and IL-12-IFN-gamma axis responses during viral infections.** C. Biron. Brown Univ.**178 11:05 Role of Toll-like receptors in innate immune responses to mycobacteria.** M.J. Fenton. Boston Univ. Sch. of Med.**11:30 Cytokine inhibitors encoded by poxviruses.** G. McFadden. Univ. of Western Ontario.**11:55 Distinct modes of CD4 gene silencing during T cell development.** D.R. Littman*. NYU Med. Ctr.**12:20 Lunch (on own)****1:15-2:00 PM SOCIETY BUSINESS MEETINGS**

SLB (South Pacific Ballroom)

ICS (Jade/Plumeria/Maile)

2:00-3:35 PM PLENARY SESSION IV: CHEMOKINES

(Jade/Plumeria/Maile)

*Chair: A. Richmond***179 2:00 CCR3 in asthma: role of the mast cell.** C. Gerard. Harvard Univ.**180 2:25 Chemokine-recruited DCs link inflammation and immunity.** K. Matsushima, H. Yoneyama and K. Matsuno. Dokkyo Univ. Sch. of Med., Japan.**2:50 Immunoregulatory functions of homeostatic chemokines and their receptors.** M. Lipp. Max Delbrück Ctr. for Molec. Med., Berlin-Buch.**181 3:15 Genetic analysis of chemokine roles in human disease.** P.M. Murphy. NIAID, NIH.**3:40 Break****4:00-6:20 PM CONCURRENT SYMPOSIA 4-6****4:00-6:15 PM SYMPOSIUM 4: CHEMOKINES**

(Jade/Plumeria/Maile)

*Cochairs: J. Oppenheim and P. Murphy***182 4:00 Resistance to septic peritonitis in mice lacking CC chemokine receptor 8 via augmenting innate immune response.** A. Matsukawa, N.W. Lukacs, C.M. Hogaboam, S.L. Kunkel and S. Lira. Kumamoto Univ. Sch. of Med., Japan; Univ. of Michigan Med. Sch. and Schering-Plough Res. Inst.**183 4:15 Chemokine receptor polymorphism and risk of acute rejection in human renal transplantation.** R. Abdi, T.T.B. Huong, A. Sahagun-Ruiz, P.M. Murphy, B.M. Brenner, E.L. Milford and D.H. McDermott. Brigham and Women's Hosp. and NIAID, NIH.**184 4:30 Hsc70 interacting protein associates with CXCR2 and regulates the receptor signaling and trafficking.** G-H. Fan, W. Yang and A. Richmond. DVA Med. Ctr., Nashville and Vanderbilt Univ. Sch. of Med.**185 4:45 Induction of chemokine CXCL11/ β -R1 by IFN- β requires PI3K.** M.R.S. Rani, N. Sizemore, G.R. Stark and R.M. Ransohoff. Cleveland Clin. Fndn.**5:00 Break****186 5:15 Interleukin-8-mediated angiogenesis by enhanced endothelial cell survival, proliferation and upregulation of MMPs production.** A. Li, S. Dubey, M.L. Varney and R.K. Singh. Univ. of Nebraska Med. Ctr.**187 5:30 TNF- α mediates SDF-1 α -induced NF- κ B activation, chemokine induction and cytotoxic effects in primary astrocytes.** Y. Han, T. He, D. Huang, C.A. Pardo and R.M. Ransohoff. Cleveland Clin. Fndn.**188 5:45 Antibacterial peptides human β -defensin-2 and LL-37 act as chemotactic factors for mast cells through distinct receptors mediating pertussis toxin-sensitive signaling.** F. Niyonsaba, K. Iwabuchi, Y. Tsutsumi-Ishii, M. Hirata, H. Ogawa and I. Nagaoka. Juntendo Univ. Sch. of Med. and Matsuzono Pharm., Iwate, Japan.**189 6:00 Induction of neutrophil IL-8 by fibrinogen and fMLF or LTB₄.** D.B. Kuhns and J.I. Gallin. SAIC Frederick, MD and NIAID, NIH.**4:00-6:00 PM SYMPOSIUM 5: INNATE IMMUNITY**

(South Pacific Ballroom)

*Cochairs: L. McPhail and M. Fenton***190 See Symposium 1, 2:35 PM, page 2.****191 4:00 Particulates can induce macrophage lineage survival and proliferation: implications for the chronicity of inflammatory responses and adjuvant action.** J.A. Hamilton. Univ. of Melbourne.**192 4:15 RelB regulates the differentiation of innate immune cells.** D. Elewaut, R. Shaikh, A.J. Leishman, E. Dejardin, H. De Winter, O. Naidenko, O. Turovskaya, D. Lo, C.F. Ware, H. Cheroutre and M. Kronenberg. La Jolla Inst. for Allergy and Immunol. and Digital Gene Technol.**193 4:30 Granulocyte colony stimulating factor promotes firm adhesion of PMN to endothelial cells.** A. Chakraborty, E.R. Hentzen, C.W. Smith. Baylor Col. of Med.**194 4:45 ULBPS, novel MHC class I-related proteins, bind to NKG2D and activate multiple signaling pathways in primary NK cells.** C.L. Sutherland, J. Chalupny, K. Schooley, T. VandenBos, M. Kubin and D. Cosman. Immunex Corp., Seattle.

* Member of US-Japan Immunology Board.

5:00 **Break**

- 195 5:15 **Toll-like receptor 2 (TLR2) and TLR4 differentially activate human dendritic cells.** F. Re and J.L. Strominger. Dana-Farber Cancer Inst. and Harvard Univ.
- 196 5:30 **Regulation of neutrophil production in adhesion molecule-deficient mice by IL-17 and G-CSF.** K. Ley and S.B. Forlow. Univ. of Virginia.
- 197 5:45 **CD14 and Toll 4 play no role in the response to encapsulated gram-negative bacteria.** S. Awasthi, E. Denamur, A. Haziot and S.M. Goyert. Hosp. Robert Debré, Paris and North Shore Univ. Hosp. Manhasset, NY.

4:00-6:20 PM **SYMPOSIUM 6: INFECTIOUS DISEASES**

(Puakeniken I & II)

Cochairs: S. Goyert and G. Poli

- 198 4:00 **Differential signaling and replication of HIV strains infecting T lymphocytes via CCR5, CXCR4, or both co-receptors.** G. Poli. San Raffaele Sci. Inst., Milan.
- 199 4:20 **Lymphotoxins control cytomegalovirus through regulation of interferon gene expression, an example of host-virus detente.** C.A. Benedict, T. Banks, M. Ko, A. Angulo, P. Ghazal and C.F. Ware. La Jolla Inst. for Allergy and Immunol., Scripps Res. Inst. and Univ. of Edinburgh.
- 200 4:35 **Stimulation via CD40 can substitute for CD4 T cell function in preventing reactivation of a latent herpesvirus.** S.R. Sarawar, B.J. Lee, S.P. Schoenberger and S.K. Reiter. La Jolla Inst. for Allergy and Immunol.
- 201 4:50 **IFN- β is critical for a host immune response to viral or tumor challenge.** E. Fish, R. Deonarain, D. Smith, D. Gewert, A. Porter, F. Dawood and P. Liu. Toronto Gen. Res. Inst. and Univ. of Toronto and Univ. of London, UK.
- 5:05 **Break**
- 202 5:20 **The cytokine inducer murabutide inhibits the expression of a novel cellular RNA helicase necessary for HIV replication.** G. Bahr, C. Cocude, O. Billaut-Mulot, M.J. Truong and C. Capron. Pasteur Inst. of Lille.
- 203 5:35 **Signaling pathways and gene expression in HIV-1-infected macrophages.** N. Vázquez, T. Wild and S.M. Wahl. NIDCR, NIH.
- 204 5:50 **A new strategy is needed to cure chronic HIV infection.** K.A. Smith, D. Warren, P. Bellman, A.M. Dunne and M. Lobo. Weill Med. Col. of Cornell Univ.
- 205 6:05 **Experimental African trypanosomiasis: interleukin-10 is crucial for survival.** H. Tabel, M. Shi and W. Pan. Univ. of Saskatchewan.

6:30-8:00 PM **POSTER SESSION II AND EXHIBITS (no host bar)**

(Haku/Pikake I & II)

Poster board numbers are indicated in bold.

Genetic Determinants of Host Resistance

- 206 **1** **Distribution of inducible nitric oxide synthase haplotypes in U.S. beef cattle.** C.G. Chitko-McKown, W.W. Laegreid and M.P. Heaton. USDA, Clay Ctr., NE.

Host-Microbe Interactions

- 207 **2** **Uroepithelial cells require TLR4, CD14 and proteases for induction of proinflammatory responses when exposed to LPS.** F. Bäckhed, S. Normark and A. Richter-Dahlfors. Karolinska Inst.
- 208 **3** **Differential bactericidal functions stimulated by the activation of Toll-like receptors in chicken heterophils.** C.L. Swaggerty, M.B. Farnell and M.H. Kogut. USDA, College Station, TX and Texas A&M Univ.
- 209 **4** **Regulation of proinflammatory cytokines in human lung epithelial cells by *Mycoplasma pneumoniae* infection.** J. Yang, W.C. Hooper, D. Phillips and D.F. Talkington. Ctrs. for Dis. Control and Prevent.
- 210 **5** **The role of galectin-3 in resistance against tumor metastasis and infection.** A. Ghaffar, M. Nachtigal and E.P. Mayer. Univ. of South Carolins Sch. of Med.
- 211 **6** **Activation of innate immune responses by intracellular pathogens.** P.A. Darrah and D.M. Mosser. Univ. of Maryland College Park.
- 212 **7** **The response of Tlr4-receptor-positive cells to pulmonary infection.** M.L. Hart, D.A. Mosier and S.K. Chapes. Kansas State Univ.
- 213 **8** **IFN- γ primed macrophages control *Salmonella typhimurium* replication by activating the MEK/ERK kinases and NADPH oxidase.** C.M. Rosenberger and B.B. Finlay. Univ. of British Columbia.
- 214 **9** **An impaired interferon gamma signaling pathway in leishmania-infected human macrophages is associated with the induction of suppressor of cytokine signaling-3.** S. Bertholet, H.L. Dickensheets, R.P. Donnelly, D. Sacks and R.T. Kenney. FDA, Bethesda and NIAID, NIH.
- 215 **10** **Leishmania species differentially modulates macrophage migration and expression of cell adhesion molecules.** S. Mendez, Y. Belkaid, E. Flowers and D.L. Sacks. NIAID, NIH.

Host-Mycobacterial Interactions

- 216 **11** **Expression of iron transport protein mRNA in murine macrophages were differentially regulated by *Mycobacterium avium* infection.** W. Zhong, W.P. Lafuse and B.S. Zwilling. Ohio State Univ.
- 217 **12** ***Mycobacterium avium* regulation of macrophage cytokine production.** T. Greenwell-Wild, N. Vázquez, D. Sim and S.M. Wahl. NIDCR, NIH.

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ICS, ISICR, ECI, and SLB
October 6-11, 2002**

- 218 **13 Roles of free fatty acids in expression of the antimicrobial activity of macrophages against *Mycobacterium tuberculosis*.** H. Tomioka, T. Akaki, T. Shimizu, K. Sato, C. Sano and K. Sano. Shimane Med. Univ., Japan.
- 219 **14 Cell-to-cell contact-dependent expression of the suppressor activity of *Mycobacterium avium* complex-induced immunosuppressive macrophages against target T cells.** T. Shimizu, H. Tomioka, K. Ogasawara, C. Sano and K. Sato. Shimane Med. Univ., Japan.
- 220 **15 A single vaccination with protein-microspheres elicits a strong CD8 T-cell-mediated immune response against *Mycobacterium tuberculosis* antigen Mtb8.4.** J.T. Evans, J.R. Ward, M.R. Alderson, J. Kern and M. Johnson. Corixa Corp., Hamilton, MT.

Infectious Diseases

- 221 **16 A limulus anti-LPS factor-derived peptide modulates cytokine gene expression and promotes resolution of bacterial acute infection in mice.** M.G. Vallespi, J.C. Alvarez-Obregon, T. Montero, H. Garay, O. Reyes and M.J. Arana. Ctr. for Genet. Engin. and Biotechnol., Havana.
- 222 **17 IL-12 increase during neutropenia in established infections.** B. Herndon, S. Latcham and D. Bamberger. Univ. of Missouri-Kansas City Sch. of Med.
- 223 **18 The immune response during persistent salmonella infection.** C. Johansson, M. Börzén and M.J. Wick. Lund Univ., Sweden.
- 224 **19 Immune mechanisms of protection from rotavirus challenge.** K.L. Warfield, S.E. Blutt, M.K. Estes and M.E. Conner. Baylor Col. of Med.
- 225 **20 Cytokine predictive values of bacteremia and mortality in the elderly with fever.** L. Marti, A. Moreno, M. Almela, X. Filella, J.L. Marín, M. Sanchez and J.M. Gatell. Hosp. Clin., Barcelona.
- 226 **21 Inhibition of IL-12 production by hepatitis C viral proteins correlates with reduced APC function of dendritic cells from HCV-infected patients.** A. Dolganiuc, K. Kodys, A. Kopasz, P. Mandrekar and G. Szabo. Univ. of Massachusetts Med. Sch.

AIDS

- 227 **22 CCR5 variants permissive for HIV-1 infection show distinct functional responses to CCL3, CCL4 and CCL5.** J.A. Turpin, H.F. Dong, O.M.Z. Howard, D. Halverson, M. Carrington, M. Dean, C. Osterling and J.J. Oppenheim. SAIC Frederick, NCI-Frederick and Southern Res. Inst., Frederick, MD.
- 228 **23 Multiplexed cytokine assay of serum from HIV-infected patients with differing disease presentations.** J.L. Rossio, D. Whitby, K. Hallett, R. Ryan, L.O. Arthur and J.D. Lifson. NCI-Frederick, SAIC Frederick, MD and Linco Res. Inc., Charles, MO.

- 229 **24 Elevated inflammation-related transcripts in HIV-infected individuals are decreased after administration of 16- α -bromoepiandrosterone (HE2000): an immunostimulatory steroid.** C. Reading, G. Khoury, T. Giese and J. Frincke. Hollis-Eden Pharmaceut. Inc. San Diego, Natl. Inst. of Virol., Johannesburg and Univ. of Heidelberg.

Viral-Cytokine Mechanisms

- 230 **25 Viral infection induces Toll-like receptor gene expression.** M. Miettinen, T. Sareneva, I. Julkunen and S. Matikainen. Natl. Publ. Hlth. Inst., Helsinki.
- 231 **26 Variations in serum IL-7 and 90K/MAC-2 binding protein levels analysed in cohorts of HIV-1 patients and correlated with clinical changes following antiretroviral therapy.** G.M. Bahr, E. Darcissac, V. Vidal, X. De La Tribonniere and Y. Mouton. Pasteur Inst. of Lille and Hosp. Ctr. of Tourcoing, France.
- 232 **27 Oral interferon therapy and the innate/acquired axis.** M.W. Beilharz, E. Bosio and A.L. Seidler. Univ. of Western Australia.
- 233 **28 HPV 16 E6 binds to TRADD and blocks activation of the downstream apoptotic pathway.** P. Duerksen-Hughes and M. Filippova. Loma Linda Univ. Sch. of Med.
- 234 **29 Light interferes with the HVEM-mediated route of herpes simplex-1 infection.** J. Whitmire, D. Mauri, R. Eisenberg, G. Cohen, P. Spear and C. Ware. La Jolla Inst. for Allergy and Immunol., Univ. of Pennsylvania and Northwestern Univ., Chicago.

Host-HIV Interactions

- 235 **30 Double edged effect of V γ 9/V δ 2 T lymphocytes on viral expression in an *in vitro* model of HIV-1/*M. tuberculosis* coinfection.** P. Biswas, M. Ferrarini, B. Mantelli, C. Fortis, D. Resta, G. Poli, A. Lazzarin and A.A. Manfredi. San Raffaele Sci. Inst., Milan.
- 236 **31 Involvement of Bcl-2 and the IL-2 receptor in HIV+ patients whose CD4 counts fail to increase significantly in response to HAART.** J. Thèze, H. Keller, L. Naït-Ighil, M-P. Treilhou, M. Joussemet, B. GaDupont, B. Gachot, J. Maral and D. David. Pasteur Inst. and Chiron-Europe, Amsterdam.
- 237 **32 Novel mechanism for T cell apoptosis via CD28 and protein kinase C activation.** D.E. Lewis, M. Merched-Sauvage and D.N. Tang. Baylor Col. of Med.

Lymphoid Cell Growth and Differentiation

- 238 **33 Characterization of a novel Ly49 promoter that is active in immature cells.** S.K. Anderson, R. Nalewaik, A. Makrigiannis and A. Saleh. NCI-Frederick, MD.
- 239 **34 Anti-IL-4 and anti-IL-13 specific hybridoma cells generated in cytokine-deficient mice show absolute dependence of IL-6 and produce low affinity IgM κ monoclonal antibodies.** V.P. Shichkin. Univ. of Cincinnati Med. Ctr.

- 240 **35** **Role of cytokine and intracellular adapter protein in the B cell development and differentiation.** K. Takatsu and S. Takaki. Univ. of Tokyo.
- 241 **36** **Role of infiltrating neutrophils in the thymus after whole-body X-irradiation.** Y. Kobayashi, T. Uchimoto, N. Watanabe, E. Kubo and M. Muto. Toho Univ. and Natl. Inst. of Radiol. Sci., Chiba, Japan.

Hematopoietic Cell Growth and Differentiation

- 242 **37** **Stimulatory effect of enterocyte-derived GM-CSF on proliferative activity of haematopoietic precursors *in vitro*.** V.V. Temchura, S.V. Sennikov, T.V. Injelevskaya, V.A. Trufakin and V.A. Kozlov. Inst. of Clin. Immunol. and Inst. of Physiol., Russian Acad of Med. Sci., Novosibirsk.
- 243 **38** **Serial analysis of gene expression in human monocyte-derived dendritic cells.** S-i. Hashimoto, S. Nagai, S. Ishikawa and K. Matsushima. Sch. of Med., Univ. of Tokyo.
- 244 **39** **Stat5-independence and altered signal transduction cascades in chronic myelogenous leukemia cells selected for Gleevec resistance.** N. Donato, J. Wu, B. Aggarwal and M. Talpaz. Univ. of Texas MD Anderson Cancer Ctr.

Lymphoid-Mediated Angiogenesis

- 245 **40** **Macrophage-tumor cell interaction in regulation of tumor angiogenesis.** R.K. Singh, M.L. Varney, A. Li and R.L. Mosley. Univ. of Nebraska Med. Ctr.

Antigen Processing and Presentation

- 246 **41** **Physical and functional loss of NKT cells following a vaccinia virus infection.** T.J. Roberts and R.R. Brutkiewicz. Indiana Univ. Sch. of Med. and Walther Oncol. Ctr., Indianapolis.
- 247 **42** **A single chain Fv anti-CD64:ovalbumin fusion protein augments antigen presentation and results in higher IgG2a production.** T.H. Sulahian, A. Sun, R.E. Symmes, J. Goldstein, K. Wardwell, R. Moser and P.M. Guyre. Dartmouth Med. Sch. and Medarex Inc., Annandale, NJ.

Leukocyte-Endothelial Interactions

- 248 **43** **IL-2 controls O-glycan branching and selectin ligand formation in CD8 T cells.** H.J. Ziltener, D.A. Carlow, S.Y. Corbel and M.J. Williams. Univ. of British Columbia.
- 249 **44** **Selective inhibition of endothelial activation by interleukin-10.** T.J. Lisinski and M.B. Furie. SUNY at Stony Brook.

Cell-Matrix Interactions

- 250 **45** **Galectin-3 binds to gelatinase B from human neutrophil leukocytes.** M. Hedlund, A. Karlsson, C. Dahlgren and H. Leffler. Lund Univ. and Univ. of Göteborg, Sweden.
- 251 **46** **Modification of bone marrow environment by TGF- β enhances survival of the leukemic cells.** J.D. Schwarzmeier, M. Shehata, R. Hubmann and M. Hilgarth. Univ. of Vienna.

Cell-Cell Interactions in the Immune Response

- 252 **47** **Effect of mouse uterine stromal cells on epithelial cell TNF- α and TGF β production and transepithelial resistance in culture.** K.S. Grant and C.R. Wira. Dartmouth Med. Sch.
- 253 **48** **Effect of rapamycin on the cyclosporin A-resistant CD28-mediated co-stimulatory pathway.** P. Ghosh, M.A. Buchholz, S. Yano and D.L. Longo. NIA, NIH, Baltimore.
- 254 **49** **Feline immunodeficiency virus infection induces B7⁺CTLA4⁺ T cell apoptosis: a model for T cell depletion and immunodeficiency.** M.B. Tompkins, M.E. Bull, J.L. Dow and W.A.F. Tompkins. Col. of Vet. Med., North Carolina State Univ.
- 255 **50** **B7.1/B7.2 and CTLA4 expression on feline T cells *in vitro* correlates with apoptosis.** T.W. Vahlenkamp, M.E. Bull, J.L. Dow, D. Anderson, M.B. Tompkins and W.A.F. Tompkins. Col. of Vet. Med., North Carolina State Univ.
- 256 **51** **T cell apoptosis in FIV-infected cats is blocked by the addition of antibodies to B7.1 and B7.2.** M.E. Bull, T.W. Vahlenkamp, M.B. Tompkins and W.A.F. Tompkins. Col. of Vet. Med., North Carolina State Univ.

Th1/Th2 Cytokines

- 257 **52** **Circulating T cells in individuals living in areas with high prevalence of infections are poised to produce Th2 cytokines.** K. Kemp, B.D. Akanmori and L. Hviid. Copenhagen Univ. Hosp., Univ. of Copenhagen and Univ. of Ghana.
- 258 **53** **Different therapeutic responses after anti-interferon-gamma therapy in Th1 (rheumatoid and psoriatic arthritis) and Th1/Th2 disease (systemic lupus erythematosus).** G. Loukina, Y. Sigidin, B. Skurkovich and S. Skurkovich. Inst. of Rheumatol., Moscow and Adv. Biotherapy Labs. Rockville, MD.
- 259 **54** **Detection of intracellular cytokines production in peripheral blood CD3⁺T cells of patients with recurrent genital herpes.** Q. Qian. Ctr. for STD Control and Res., Shenzhen, China.
- 260 **55** **IFN- α and IL-12 induce interferon regulatory factor 4 (IRF-4) and IRF-8 gene expression in human NK and T cells.** A. Lehtonen, R. Lund, T. Sareneva, R. Lahesmaa, I. Julkunen and S. Matikainen. Natl. Publ. Hlth. Inst., Helsinki and Turku Ctr. for Biotechnol., Finland.
- 261 **56** **A proteome database of human primary T helper cells.** T.A. Nyman, A. Rosengren and R. Lahesmaa. Turku Ctr. for Biotechnol., Finland.
- 262 **57** **Serum level of the main immunoregulation cytokines and some immunological status parameters in the population exposed to radiation influence resulted from the nuclear test action at the Semipalatinsk test site.** L.V. Grishina, Y.A. Sennikova, S.V. Kisselev, E.L. Gelfgat, S.V. Krysov, N.Y. Soloveva, S.V. Sennikov and V.A. Kozlov. Inst. of Clin. Immunol. SB RAMS, Novosibirsk, Russia.

263 **58 IL-4 administration restores immunity in male mice following ethanol exposure and burn injury.** K.A.N. Messingham, S.A. Heinrich and E.J. Kovacs. Loyola Univ. Chicago.

264 **59 Circulating anti-human heat shock protein 65 IgE autoantibody in atopic dermatitis.** T-E. Kim and G. Noh. FoodBioTech Co., Seoul.

265 **60 Synergistic effect of IL-4 on IL-2- and IL-12-induction of murine IFN- γ expression in NK cells.** J.H. Bream, C. Rong-Yu, R. Curiel, M. Grusby, T. Aune and H.A. Young. NCI-Frederick, MD.

266 **61 Th1/Th2 cytokine expression by cord blood mononuclear cells compared to adult PBMCs: the sensitive measurement by a novel cell-based ELISA assay.** H.R. Morse, V. Laundry and J.M. Hows. Univ. of Bristol, Southmead Hosp., UK.

Inflammation

267 **62 The neuroinflammatory role of CAP37.** H.A. Pereira, X. Ruan, D. Williams and P. Kumar. Univ. of Oklahoma Hlth. Sci. Ctr.

268 **63 Upregulation of IL-18 and IL-12 in the ileum of rats with necrotizing enterocolitis.** B. Dvorak, M.D. Halpern, H. Holubec, J.A. Dominguez, C.S. Williams, Y.G. Meza and D.L. McWilliam. Univ. of Arizona.

269 **64 Anti-inflammatory effect of newly synthesized tumor necrosis factor- α , D-297.** E-S. Yoo, H.K. Kang, J.S. Park and J.Y. Cho. Cheju Natl. Univ. Med. Sch. and Daewoong Pharm. Co. Ltd., Sungnam, Republic of Korea.

270 **65 Endotoxemia induces rapid shedding followed by upregulation of the monocyte hemoglobin scavenger receptor CD163.** K.A. Hintz, A.J. Rassias, K. Wardwell, J.I. Goldstein, K.E. Goonan, T.H. Sulahian, A.L. Givan, P.K. Wallace, M.P. Yeager, P.M. Guyre. Dartmouth Med. Sch. and Dartmouth-Hitchcock Med. Ctr.

271 **66 A role for interferon- γ in controlling neutrophil recruitment during peritoneal inflammation.** R.M. McLoughlin, T.S. Wilkinson, S.A. Jones and N. Topley. Cardiff Sch. of Biosci., Cardiff Univ.

272 **67 Regulation of leukocyte recruitment during acute inflammation by IL-6 and its soluble receptor.** S.M. Hurst, T.S. Wilkinson, R.M. McLoughlin, S. Horiuchi, N. Yamamoto, G.M. Fuller, N. Topley and S.A. Jones. Cardiff Sch. of Biosci., Cardiff Univ., Univ. of Wales Col. of Med., Tokyo Med. and Dent. Univ. and Univ. of Alabama at Birmingham.

273 **68 Oncostatin-M: a differential regulator of chemokine expression.** S.M. Hurst, J. Monslow, R.M. McLoughlin, N. Topley and S.A. Jones. Cardiff Sch. of Biosci., Cardiff Univ. and Univ. of Wales Col. of Med.

274 **69 IFN gamma depletion enhances inflammation and lesion progression of *Leishmania major*-infected IL-4-deficient C57BL/6 mice.** E. Caler, N. Noben-Trauth, D. Sacks and Y. Belkaid. NIAID, NIH.

275 **70 Altered inflammatory response in TSG-14/PTX-3 transgenic mice.** A.A.M. Dias, D.G. Souza, S.N. Diniz, R.N. Gomes, A. Goodman, P.T. Bozza, A.L. Montagnini, J. Vilcek, M.M. Teixeira and L.F.L. Reis. Ludwig Inst. for Cancer Res. São Paulo, UFMG, Belo Horizonte, Brazil, FioCruz, Rio de Janeiro, NYU Sch. of Med. and Cancer Hosp., São Paulo.

276 **71 Anti-IL-6 receptor antibody treatment improves interstitial lung diseases in IL-6 transgenic mice.** Y. Sugamata, H. Nakahara, A. Katsume, M. Sugimoto, K. Yoshizaki and N. Nishimoto. Sch. of Hlth. and Sport Sci., Osaka Univ.

Immunopathologic Mechanisms

277 **72 Antigen dose defines opposite Th1/Th2-type responses in the lungs of C57BL/6 and BALB/c mice independently of splenic responses.** T. Morokata, J. Ishikawa and T. Yamada. Yamanouchi Pharmaceut. Co. Ltd., Ibaraki, Japan.

278 **73 Transplant related ischemia, HIF-1 α , and fibrogenic growth factors.** C.C. Baan, J.N.M. Ijzermans, T. van Gelder, A.M.A. Peeters, W.M. Mol, H.G.M. Niesters and W. Weimar. Univ. Hosp. Rotterdam, The Netherlands.

279 **74 Estrogen replacement restores immunity in aged mice by suppressing IL-6 production.** E.J. Kovacs, L.a. Duffner, P.L. Witte and K.A.N. Messingham. Loyola Univ. Med. Ctr.

280 **75 Food-specific oral tolerance induction for milk using interferon- γ in atopic dermatitis.** G. Noh. Seoul Allergy Clin.

281 **76 Mechanisms of hemorrhage-induced neutrophil priming for acute lung injury: a role for MIP-2.** J.L. Lomas, C.S. Chung, G.Y. Song, P.S. Grutkoski, A.L. Dunican, H.H. Simms and A. Ayala. Rhode Island Hosp., Providence.

Immune Deficiencies

282 **77 In vivo NK cells activation during bacterial infection in a patient with severe combined immunodeficiency.** C. Alonso, F. Borrego, R. Solana and J. Peña. Reina Sofia Hosp., Univ. of Cordoba, Spain.

Autoimmune States

283 **78 Possible roles of aberrant expression of B lymphocyte chemoattractant (BLC/CXCL13) in breakdown of immunological tolerance and autoantibody production in murine lupus.** S. Ishikawa, T. Sato, M. Abe, S. Nagai, N. Onai, H. Yoneyama, Y-y. Zhang, T. Suzuki, S-i. Hashimoto, T. Shirai, M. Lipp and K. Matsushima. Sch. of Med., Univ. of Tokyo, Juntendo Univ. Sch. of Med. and Niigata Univ. Sch. of Med., Japan and Max Delbrück Ctr. for Molec. Med., Berlin.

284 **79 IL-18 and IL-18BP have differing effects on the induction or recovery phase of the DSS model of colitis.** F.R. Byrne, H.L. Brown, S. Flores, D. Danilenko, R. Faggioni and G. Senaldi. Amgen Inc.

285 **80 Cytokine stimulation by developmental anti-gens, a potential mechanism for autoimmune disease.** M.A. Berman, L-Q. Tai, K.L. Imfeld, F. Zaldivar and D.K. McCurdy. Children's Hosp. of Orange County, CA.

- 286 **81** **Suppression of autoimmune arthritis in IL-1-deficient mice in which T cell activation is impaired due to low levels of CD40L and OX40 expression on T cells.** S. Saijo and Y. Iwakura. Univ. of Tokyo.

Immunomodulators/Immunoregulation

- 287 **82** **Regulation of IFN-gamma gene expression in mouse peritoneal macrophage by cytokines.** S. Gessani, P. Puddu, B. Bongiovanni, L. Fantuzzi, M. Del Cornò and F. Belardelli. Ist. Superiore di Sanità, Rome.
- 288 **83** **Engagement of the phosphatidylserine receptor on dendritic cells inhibits their maturation by a TGFbeta-independent mechanism.** P.R. Hoffmann, V. Fadok and P.M. Henson. Natl. Jewish Med. and Res. Ctr.
- 289 **84** **Interleukin-1 stimulates wound healing and local immunity in patients with skin trophic ulcers.** A. Simbirtsev, E. Variouchina, A. Bubnov, V. Moskalenko and S. Ketlinsky. Inst. of Highly Pure Biopreparations, St. Petersburg, Russia.
- 290 **85** **The soluble form of the IL-1 receptor accessory protein induces a bi-phasic response upon IL-1 stimulation.** R.L. Smeets, F.A.J. van de Loo, H.M. Beuningen, I. Dmitriev, D.T. Curiel, O. Rosati, M.U. Martin, L.A.B. Joosten and W.B. van den Berg. Univ. Med. Ctr. Nijmegen, The Netherlands, Univ. of Alabama at Birmingham and Hannover Med. Sch., Germany.
- 291 **86** **Expression of functional P2z/P2X7 purinergic receptor by rat alveolar macrophages and its modulation by cytokines.** I. Lemaire and N. Leduc. Univ. of Ottawa.
- 292 **87** **ASF as a novel immunoregulator.** T.S. Davidson and W.F. Hickey. Dartmouth Med. Sch.
- 293 **88** **Oral ascorbic acid and beta-glucan affect cytokine expression differently after an LPS challenge.** S.D. Eicher, C.A. McKee, J.A. Carroll and T.R. Johnson. USDA, West Lafayette, IN and Columbia, MO and Purdue Univ.
- 294 **89** **Effects of androstenetriol on microscopic lesions of ulcerative colitis in a rat model of inflammatory bowel disease.** B. Richard, C. Meschter, C. Reading, U. Orlinska and C. Ahlem. Hollis-Eden Pharmaceut., San Diego and Comparative Biosci., Santa Clara, CA.
- 295 **90** **Administration of β -AET to burned mice modifies bone loss, structure and dynamics.** U. Orlinska, B. Richard, C. Ahlem, C. Reading, J. Shelby and S. Miller. Hollis-Eden Pharmaceut. Inc., San Diego and Univ. of Utah.
- 296 **91** **Increased IL-10 and decreased IL-12 production correlate with reduced antigen presentation in alcohol-exposed myeloid dendritic cells.** P. Mandrekar, D. Catalano and G. Szabo. Univ. of Massachusetts Med. Ctr.

Immunopharmacology

- 297 **92** **Apoptosis-inducing activity of novel triterpene saponins, securiosides A and B against M-CSF-stimulated macrophages.** S. Yui, Y. Mimaki, Y. Sashida and M. Yamazaki. Fac. of Pharmaceut. Sci., Teikyo Univ. and Sch. of Pharm., Tokyo Univ. of Pharm. and Life Sci.

- 298 **93** **Inhibition of macrophage migration inhibitory factor tautomerase and biological activity by the acetaminophen metabolite, NAPQI.** P.D. Senter, Y. Al-Abed, C.N. Metz, F. Benigni, R.A. Mitchell, J. Chesney, J. Han, C.G. Gartner, S.D. Nelson and R. Bucala. Cytokine PharmaSci. Inc., Seattle, Picower Inst. for Med. Res., Manhasset, NY and Univ. of Washington.

Immunotherapy

- 299 **94** **Anti-human interferon-gamma antibodies in the treatment of patients with corneal transplant rejection.** S. Skurkovich, A. Kasparov, N. Narbut and B. Skurkovich. Adv. Biotherapy Labs., Rockville, MD, Sci. Res. Inst. of Eye Dis. of Russian Acad. of Sci., Moscow and Brown Univ.
- 300 **95** **Tumoricidal activation of macrophages and effector cells by liposome-encapsulated (MLV) CGP31362 plus IL-2: rapid release of interferon gamma and inflammatory cytokines.** J.J. Killion and G. Nelkin. Univ. of Texas MD Anderson Cancer Ctr.
- 301 **96** **Construction of MAFJ6-1 DNA vaccine for immunotherapy of tumors.** M-H. Wang, G. Li, Y-M. Lin, Y-H. Song and K-F. Wu. Inst. of Hematol., Chinese Acad. of Med. Sci. and Peking Union Med. Col.
- 302 **97** **Correction of genetically determined low- and nonresponsiveness to hepatitis B surface antigen by vaccination with a mixture of alum-adsorbed HBsAg and alum-adsorbed cytokines.** M. Teller, K. Melber, Z. Janowicz and F.W. Falkenberg. Ruhr Univ. Bochum and RheinBiotech GmbH, Düsseldorf.
- 303 **98** **Tumor vaccines containing alum-adsorbed IL-2 as an adjuvant stimulate protective cellular immune responses.** M. Peters and F.W. Falkenberg. Ruhr Univ. Bochum.
- 304 **99** **Anti-tumor effects of liposomal IL-2 in a MCA 38 colon liver metastases tumor model.** M.E. Neville, C. Hubbard, R.J. Robb and M.C. Popescu (Spon: M.H. Ryan). Biomira USA Inc., Cranbury, NJ.
- 305 **100** **The kinetics of an IFN gamma response.** W.D. Culp, Jr., A. Perez-Diez, R.D. Massey and P. Matzinger. NIAID, NIH.
- 306 **101** **Optimization of molecular adjuvants for vaccines: SP1017 copolymer enhances expression of cytokine genes in muscle.** M.J. Turk, M-A. Perales, P. Lemieux, A.N. Houghton, K. Tsang, G. Fantuzzi and J.D. Wolchok. Mem. Sloan-Kettering Cancer Ctr., Supratek Pharma Inc., Quebec and Univ. of Colorado Hlth. Sci. Ctr.
- 307 **102** **IL-12 and IL-18 DNA: molecular adjuvants for antitumor vaccines.** C.R. Ferrone, M-A. Perales, J.D. Wolchok, T. Ramirez-Montagut, P. Gregor, G. Fantuzzi and A.N. Houghton. Mem. Sloan-Kettering Cancer Ctr. and Univ. of Colorado Hlth. Sci. Ctr.

Diagnostics/Experimental Therapies

- 308 **103** Comparison of cytokine measurements by ELISA with cytokine mRNA estimations by quantitative RT-PCR: is there a correlation between the two? A.M. Yates, C.J. Turner, O.R. Jones, E.D. Williamson and M.J. Pearce. DSTL CBS Porton Down, Salisbury, UK.
- 309 **104** Combination of lactam steroids with interferon-alpha for the treatment of pancreatic adenocarcinoma cells. D.T. Trafalis, D. Tsavdaridis, A. Papageorgiou, P. Stravrovadi, A. Barich, C. Camoutsis, P. Karamanakis, M. Koutsilieris, P. Ginopoulos and M. Sougleri. Univ. of Patras, Med. Sch., Univ. of Athens, 32nd Hosp. IKA, Thessaloniki, Anticancer Hosp., Thessaloniki, 51st Surg. Univ. Clin. and Gen. Hosp., Athens and Patras.
- 310 **105** Mouse cytokine multiplex bead immunoassay: correlation with traditional ELISAs. B. Shuman, O. Castilleja and K. Reagan. BioSource Intl. Inc., Camarillo, CA.
- 311 **106** Macrophage inhibitory cytokine-1 in epithelial neoplasia. D.A. Brown, T. Liu, R.L. Ward, N.J. Hawkins, W.D. Fairlie, A.R. Bauskin, P.J. Russell, D.I. Quinn, J.J. Grygiel, A.G. Moore, R.L. Sutherland, J. Turner, E.A. Kingsley and S.N. Breit. St. Vincent's Hosp. and Prince of Wales Hosp., Univ. of New South Wales, Australia.
- 312 **107** A theoretical model for the simulation of sepsis. R. Kumar, C.C. Chow, G. Clermont and Y. Vodovotz. Univ. of Pittsburgh.
- 313 **108** Withdrawn.

Oncogenesis

- 314 **109** The cytokine profile in TPA-induced tumor promotion and its inhibition by perilla. H. Ueda and M. Yamazaki. Fac. of Pharmaceut. Sci., Teikyo Univ., Japan.
- 315 **110** The global suppression of cytokine genes in prostate cancer cell lines is associated with androgen-sensitivity. L.L. Reznikov, A. van Bokhoven, J.A. Emmick, S-H. Kim, H.L. Miller, W.U. Johannes, V.V. Tryon, L.M. Glode, G.J. Mill and C.A. Dinarello. Univ. of Colorado Hlth. Sci. Ctr. and Source Precision Med. Inc., Boulder.
- 316 **111** Deregulation of Notch2 signaling in B-CLL. R. Hubmann, J. Schwarzmeier, M. Shehata, M. Hilgarth and M. D  chler. Univ. of Vienna Med. Sch.

Disease Roles of Cytokines

- 317 **112** Clinical and biological elements regarding the network of cytokines in multiple myeloma. V.M. Lauta. Univ. of Bari Med. Sch., Italy.
- 318 **113** Soluble macrophage colony-stimulating factor receptor in children hematopoietic disorders. X. Sha, Q. Rao, Y. Song and K. Wu. Inst. of Hematol., Chinese Acad. of Med. Sci., Tianjin.

- 319 **114** Adenoviral expression of murine oncostatin M induces inflammation and bone apposition in joints of IL-1, IL-6 and TNF- α -deficient mice. A.S.K. de Hooe, M. Bennink, F.A.J. van de Loo, C.D. Richards, O.J. Arntz, E. Lubberts and W.B. van den Berg. Univ. Med. Ctr., Nijmegen, The Netherlands and McMaster Univ., Canada.
- 320 **115** Dual roles of IL-6 in airway hypersensitivity suggested by enhanced mucus secretion and reduced inflammatory cell infiltration in ovalbumin-challenged IL-6-deficient mice. N. Mukaida, Z. Qiu, J. Tamura and K. Kurashima. Sch. of Med., Kanazawa Univ., Japan.
- 321 **116** Regulation of macrophage chemokine expression by hypoxia: a potential role in the acute respiratory distress syndrome. N. Hirani, S.C. Donnelly, R.M. Strieter and C. Haslett. Med. Sch., Edinburgh Univ.
- 322 **117** Neutralisation of endogenous IL-18 activity is a disease-modifying therapy in the collagen-induced model of arthritis. S. Alouani, C. Plater-Zyberk, L.A.B. Joosten, M.M.A. Helsen, P. Sattouet-Roche, C. Siegfried, F.A.J. van de Loo, P. Graber, S. Aloni, R. Cirillo, E. Lubberts, C.A. Dinarello, W.B. van den Berg and Y. Chvatchko. Sero Pharmaceut. Res. Inst., Geneva, Univ. Med. Ctr. St-Radboud, Nijmegen, The Netherlands, InterPharma Labs., Nes Ziona, Israel, Biomed. Res. Inst. Antoine Marxer, Italy and Univ. of Colorado Hlth. Sci. Ctr.
- 323 **118** Interleukin (IL)-18/IL-18 binding protein signalling modulates atherosclerotic lesion development and stability. A. Corbaz, Z. Mallat, A. Scoazec, P. Graber, S. Alouani, B. Esposito, T. Humbert, P. Henry, A. Tedgui and Y. Chvatchko. INSERM U541, Paris VII, Hosp. Lariboisi  re, Paris and Sero Pharmaceut. Res. Inst., Geneva.
- 324 **119** Production of cytokines by erythroid nuclear cells of bone marrow isolated from patients with myelodysplastic syndromes. T.V. Injelevskaya, S.V. Sennikov, V.A. Kozlov, I.B. Kovinev and M.I. Loseva. Inst. of Clin. Immunol. SB RAMS, Novosibirsk Med. Acad., Russia.
- 325 **120** Adenoviral expression of IL-10 promotes a dose-dependent survival advantage to zymosan-induced ARDS. M.E. Murday, F.R. Bahjat, R.M.R. Ungaro, J. DeBernardis and L.L. Moldawer. Univ. of Florida Col. of Med.
- 326 **121** Macrophage inflammatory protein 1 α inhibits the production of IL-4 by splenic T cells stimulated with monocyte chemoattractant protein 1. M. Kobayashi, H. Takahashi, D.N. Herndon, R.B. Pollard and F. Suzuki. Univ. of Texas Med. Br. and Shriners Burns Hosp., Galveston.
- 327 **122** Glycyrrhizin inhibits the production of monocyte chemoattractant protein 1 in cultures of T cells and macrophages. F. Suzuki, M. Takei, M. Kobayashi and R.B. Pollard. Univ. of Texas Med. Br., Galveston.
- 328 **123** Effect of macrophage inflammatory protein 1 α on the CLP-induced infectious complications in a SCID-human chimera model of thermal injury. H. Takahashi, M. Kobayashi, D.N. Herndon, R.B. Pollard and F. Suzuki. Univ. of Texas Med. Br. and Shriners Burns Hosp., Galveston.
- 329 **124** MTX and MPA are immunosuppressive by a different mechanism. S. de Lathouder, A. Gerards, E. de Groot, B. Dijkmans and L. Aarden. CLB and VU Univ. Med. Ctr., Amsterdam.

- 330 125 **Increased serum levels of interleukin-1 receptor antagonist in human obesity: a link to the resistance to leptin?** C.A. Meier, E. Bobbioni, C. Gabay, F. Assimacopoulos-Jeannet, A. Golay and J-M. Dayer. Univ. Hosp. and Univ. Med. Sch., Geneva.
- 331 126 **Evaluation of therapeutic effects of recombinant IL-1 receptor antagonist and superoxide dismutase on a model of experimental arthritis in mice.** A. Ischenko, V. Dobritsa, I. Churilova, A. Zhakhov, L. Solovieva, T. Antipova and S. Ketlinsky. Inst. of Highly Pure Biopreparations, St. Petersburg, Russia.
- 332 127 **IL-15 plays an important role in the pathogenesis of HAM/TSP through activation of the T cells and persistence of antigen-specific CD8 cells.** N. Azimi, M. Nagai, J. Mariner, S. Jacobson and T.A. Waldmann. NCI and NINDS, NIH.
- 333 128 **IL-1 α , but not IL-1 β , is required for contact allergen-specific T cell activation during the sensitization phase in contact hypersensitivity.** S. Nakae, C. Naruse-Nakajima, K. Sudo, R. Horai, M. Asano and Y. Iwakura. Univ. of Tokyo.

8:00-10:00 PM **POSTER DISCUSSIONS/WORKSHOPS**

Posters will be selected for discussions/workshops.

Sunday, November 11

7:30-8:30 AM **CONTINENTAL BREAKFAST**
(Haku/Pikake I & II)

8:30-12:20 PM **PLENARY SESSION V: CLINICAL IMPACT OF CYTOKINES**
(Jade/Plumeria/Maile)

Cochairs: K. Okumura and R. Schreiber

8:30 TBA. N.H. Ruddle. Yale Univ. Sch. of Med.

334 8:55 **TACI-Ig neutralizes molecules critical for B cell development and autoimmune disease: BLyS is required for B cell development.** J.A. Gross, S.R. Dillon, S. Mudri, J. Johnston, A. Littau, R. Roque, M. Rixon, K.P. Foley, A. Grossman and C.H. Clegg. ZymoGenetics Inc., Seattle.

9:20 **Disorders of development due to defects in the ectodysplasin-A signaling pathway.** J. Zonana. Oregon Hlth. Sci. Univ.

335 9:45 **Critical role for tumor necrosis factor-related apoptosis-inducing ligand in innate immune surveillance against tumors.** K. Okumura*. Juntendo Univ. Sch. of Med., Japan.

10:10 **Break**

336 10:40 **Modulation of chemokine signaling involves multiple receptor-associated proteins.** A. Richmond, G.H. Fan, J. Sai and D.Z. Wang. Vanderbilt Univ. Sch. of Med.

11:05 **Manipulation of the lymphotoxin system leads to many potential clinical applications.** J. Browning. Biogen Inc., Cambridge, MA.

11:30 TBA. C. Smith. Immunex Corp., Seattle.

337 11:55 **Cancer immunoediting by IFN- γ and lymphocytes.** V. Shankaran, H. Ikeda, A. Bruce, L. Old and R.D. Schreiber. Washington Univ. Sch. of Med. and Ludwig Inst. for Cancer Res., New York.

12:30-1:30 PM **SLB MARIE T. BONAZINGA AWARD LECTURE**

(Jade/Plumeria/Maile)



G. Jeanette Thorbecke

12:30 **Introduction.** S. Wahl. NIDCR, NIH.

338 12:35 **Antigen-specific T cell-mediated gene therapy with latent TGF- β 1 in the treatment of experimental allergic encephalomyelitis.** G.J. Thorbecke, L.Z. Chen and G.M. Hochwald. NYU Sch. of Med.

1:30 **Lunch (on own)**

2:30-4:45 PM **CONCURRENT SYMPOSIA 7-9**

2:45-4:45 PM **SYMPOSIUM 7: INNATE IMMUNITY**
(Jade/Plumeria/Maile)

Cochairs: S. Vogel and M. Rubenstein

339 2:30 **TLR4, but not TLR2 agonists, activates phosphorylation of STAT1 α : role of LPS-induced IFN- β .** S.N. Vogel, V. Toshchakov and M.J. Fenton. Uniformed Serv. Univ. of Hlth. Sci. and Boston Univ. Med. Ctr.

340 2:45 **Effects of acute exposure to nitrogen dioxide on human bronchial epithelial cells: involvement of nitric oxide and IL-8.** V. Ayyagari, A. Januszkiewicz and J. Nath. Walter Reed Army Inst. of Res., Silver Spring, MD.

* Member of US-Japan Immunology Board.

341 3:00 **Biasing T cell responses by manipulating antigen presenting cells.** C.F. Anderson and D.M. Mosser. Univ. of Maryland College Park.

342 3:15 **Upregulation of IL-10R1 expression is required to render human neutrophils fully responsive to IL-10.** M.A. Cassatella, L. Crepaldi, S. Gasperini, J.A. Lapinet, F. Calzetti, C. Pinardi, Y. Liu, S. Zurawski, R. de Waal Malefyt and K.W. Moore. Univ. of Verona and DNAX Res. Inst. of Molec. and Cell. Biol., Palo Alto.

3:30 **Break**

343 3:45 **Hypoxia and IFN- γ inhibit viral replication in macrophages.** L. Varesio, S. Pastorino, M. Puppo, A. Negrioli and M.C. Bosco. G. Gaslini Inst., Genoa.

344 4:00 **In vivo-derived dendritic cells show a different regulatory dependence for retaining immature function than GM-CSF differentiated bone marrow-derived dendritic cells.** M. Chattergoon and L.J. Montaner. Univ. of Pennsylvania Sch. of Med. and Wistar Inst., Philadelphia.

345 4:15 **Contrasting role of heat shock protein 27 on dendritic cell differentiation versus maturation.** A. De, K. Laudansky and C. Miller-Graziano. Univ. of Rochester Med. Ctr.

346 4:30 **Transcriptional regulation of the IL-18 binding protein gene.** M. Rubinstein, D. Novick and V. Hurgin. Weizmann Inst. of Sci., Rehovot.

2:30-4:45 PM **SYMPOSIUM 8: CYTOKINES IN PATHOGENESIS**

(South Pacific Ballroom)

Cochairs: C. Miller-Graziano and I. Campbell

347 2:30 **A role for BAFF in the development of Sjögren's syndrome through recruitment of B cells with a marginal zone-like phenotype.** F. Mackay, J. Groom, S.L. Kalled, P. Schneider, J. Tschopp, T.G. Cachero, M. Batten, J. Wheway and T.P. Gordon. Garvan Inst. of Med. Res., Darlinghurst, Australia.

348 2:45 **STAT1 protects against the neurotoxic actions of IFN- α mediated by distinct signaling mechanisms in vivo.** I.L. Campbell and J. Wang. Scripps Res. Inst.

349 3:00 **The role of the G-protein coupled receptor FPRL1 in Alzheimer's disease: its relevance to cellular uptake of β amyloid peptide and fibrillar formation by macrophages.** J.M. Wang, H. Yazawa, Z.-X. Yu, K. Takea, Y. Le, W. Gong, V.J. Ferrans, C.C. Li and J.J. Oppenheim. NCI-Frederick, SAIC Frederick, MD and NHLBI, NIH.

350 3:15 **Identification of NPI-1302a-3, an orally active TNF and IL-1 synthesis inhibitor with a novel mechanism of action.** M. Palladino, M. Hensler, B. Shlopov, A. Danks, A. Oberholzer, C. Oberholzer, T. Ling, R. Craig, C. Glembotski, E. Theodorakis and L. Moldawer. Nereus Pharmaceut. Inc., San Diego, Univ. of Florida, UCSD and San Diego State Univ.

3:30 **Break**

351 3:45 **Pulmonary inflammation induced by *Pseudomonas aeruginosa* virulence factors: role of IRF-1.** G. Fantuzzi, C.W. Wieland, B. Siegmund, M. Vasil and G. Senaldi. Univ. of Colorado Hlth. Sci. Ctr. and Amgen Inc.

352 4:00 **Protective effects of IL-18 during the acute phase of colitis: potential role for IL-11.** T.T. Pizarro, C.A. Moskaluk, K.M. Overman, B.D. Weber and B. Coruh. Univ. of Virginia Hlth. Syst.

353 4:15 **Co-dependency of tumor regression and anti-angiogenesis on both IFN- γ and Fas/FasL in mice treated with IL-12/pulse IL-2.** R.H. Wiltout, E. Gruys, J. Subleski, K. Nagashima T.C. Back and J.M. Wigginton. NCI, NIH, Frederick and Bethesda.

354 4:30 **Defective NF- κ B activation in tumor-associated macrophages.** A. Saccani, B. Bottazzi, A. Mantovani and A. Sica. Mario Negri Pharmacol. Res. Inst., Milan and Univ. of Milan.

2:30-4:45 PM **SYMPOSIUM 9: ADAPTIVE IMMUNITY** (Puakeniki I & II)

Cochairs: S. Wahl and T. Waldmann

355 2:30 **Contrasting roles of IL-2 and IL-15 in the life and death of lymphocytes: implications for immunotherapy.** T. Waldmann, N. Azimi, S. Dubois and Y. Tagaya. NCI, NIH.

356 2:45 **The B7RP-1/ICOS T cell co-stimulation pathway.** S.K. Yoshinaga, S. Khare, M. Coccia, G. Senaldi, C. Baikalov, T.W. Mak, A. Tafuri-Bladt, D. Danilenko, T. Horan, H. Goldman, H. Kohno, M. Zhang, J.S. Whoriskey, K. Gaida, M. Gresser, G. Ara and C.K. Edwards III. Amgen Inc.

357 3:00 **IL-7 and IL-15 are essential for the proliferation and survival of memory CD8 T cells.** P.V. Sivakumar, M. Glaccum, M. Kennedy, M. Bevan, C. Benoist, D. Mathis, E. Butz and A. Goldrath. Immunex Corp., Seattle, Joslin Diabetes Ctr., Boston and Univ. of Washington.

358 3:15 **Leptin: a pivotal mediator for intestinal inflammation.** B. Siegmund, H.A. Lehr, C.A. Dinarello and G. Fantuzzi. Univ. of Colorado, Hlth. Sci. Ctr.

3:30 **Break**

359 3:45 **The TNFR family member HVEM plays a role in thymic selection processes.** S. Rickert, S.W. Granger, M. Kronenberg and C.F. Ware. La Jolla Inst. for Allergy and Immunol.

360 4:00 **Overexpression of murine IL-17E induces a Th2-like response and multi-organ inflammation in transgenic mice.** G. Pan, D. French, W. Mao, M. Maruoka, P. Risser, J. Lee, J. Foster, S. Aggarwal, K. Nicholes, S. Guillet, P. Schow and A.L. Gurney. Genentech Inc.

361 4:15 **The regulatory effect of epithelial production of transgenic IL-10 on mucosal immune responses.** H. De Winter D. Elewaut, O. Turovskaya, M. Huflejt, C. Shimeld, A. Hagenbaugh, S. Binder, I. Takahashi, M. Kronenberg and H. Cheroutre. La Jolla Inst. for Allergy and Immunol.

362 4:30 **Transforming growth factor- β 1 induces anergic/suppressor CD4⁺ CD25⁺ CTLA-4⁺ T cells.** W. Chen, M. Frank, W. Jin, K.-j. Lei, N. Hardegen, S.M. Wahl. NIDCR, NIH.

5:00-8:00 PM **LUAU** (Luau Gardens)

Abstracts

ICS LIFETIME AWARD (1-2)

1

The Suppressor of Cytokine Signaling-1 (SOCS-1)

Donald Metcalf. The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

The Suppressor of Cytokine Signaling-1 (SOCS-1) is a cellular protein that is highly inducible by cytokine signaling and a member of a group of 30 proteins sharing a C-terminal SOCS-BOX region that, by binding to Elongins B and C, may target these proteins for degradation. The prototype SOCS-1 functions to terminate or suppress cytokine receptor signaling by various mechanisms according to the receptor involved. Inactivation of the SOCS-1 gene leads to death in neonatal life from fatty degeneration and necrosis of the liver, T-lymphocyte and macrophage infiltration of the pancreas, lungs and skin and reduced numbers of T- and B- lymphocytes. This disease is mediated by IFN γ and death and organ pathology can be eliminated by administration of antibodies to IFN γ or by deletion of the IFN γ gene. SOCS-1 $-/-$ mice exhibit T-cell activation and the disease may be initiated by the aberrant development of autoaggressive T cells. Mice that are SOCS-1 $-/-$ IFN γ $+/-$ die as young adults with extensive polymyositis, myocarditis and corneal infiltration and ulcer formation, diseases involving massive infiltration by T-lymphocytes, macrophages and eosinophils. SOCS-1 $-/-$ IFN γ $-/-$ mice die prematurely in the second year of life from polycystic kidney disease and chronic inflammatory disease in the lung, skin, gut and, less often, other organs. SOCS-1 does suppress signaling from many cytokine receptors and, in other contexts these may play a more important role. Thus SOCS-1 has been reported to be inactivated in a high proportion of human hepatomas.

2

Cytokine control of hematopoiesis and leukemia: From basic biology to the clinic

Leo Sachs. Weizmann Institute of Science, Rehovot, Israel

The establishment of a cell culture system for the clonal development of hematopoietic cells made it possible to discover the proteins that regulate cell viability, multiplication and differentiation of different hematopoietic cell lineages, and the molecular basis of normal and abnormal blood cell development. The first proteins discovered in this way are cytokines now called colony stimulating factors (CSFs). They also now include various other cytokines. There is a network of cytokine interactions, which has positive regulators such as CSFs and interleukins (ILs) and negative regulators such as transforming growth factor β and tumor necrosis factor. This cytokine network allows considerable flexibility and the ready amplification of response to a particular stimulus. Malignancy can be suppressed in certain types of leukemic cells by inducing differentiation with cytokines that regulate normal hematopoiesis or with other compounds that use alternative differentiation pathways. This created the basis for the clinical use of differentiation therapy. The suppression of malignancy by inducing differentiation showed that malignant cells can be reprogrammed. There is considerable plasticity in the developmental programs of normal and malignant hematopoietic cells. Different CSFs and ILs suppress programmed cell death (apoptosis) and induce cell multiplication and differentiation, and these processes of development are separately regulated. The same cytokines suppress apoptosis in normal and leukemic cells, including apoptosis induced by radiation and cytotoxic cancer chemotherapeutic compounds. An excess of cytokines can increase leukemic cell resistance to cytotoxic therapy. The tumor suppressor gene wild-type p53 induces apoptosis that can also be suppressed by cytokines. The oncogene mutant p53 suppresses apoptosis. A hematopoietic cytokine such as granulocyte CSF is now used clinically to correct defects in

hematopoiesis, including repair of radiation and chemotherapy-associated suppression of normal hematopoiesis in cancer patients, stimulation of normal granulocyte development in patients with infantile congenital agranulocytosis, and to induce migration of hematopoietic precursors from the bone marrow to peripheral blood for transplantation. Treatments that decrease the level of apoptosis-suppressing cytokines and downregulate expression of apoptosis-suppressing genes in cancer cells could improve cytotoxic cancer therapy. The basic studies on hematopoiesis and leukemia have thus provided new approaches to therapy reviewed in 1-4. 1.Sachs L. The control of growth and differentiation in normal and leukemic blood cells. The 1989 Alfred P. Sloan Prize of the General Motors Cancer Research Foundation. Cancer. 65:2196-2206, 1990. 2.Sachs L. The adventures of a biologist: Prenatal diagnosis, hematopoiesis, leukemia, carcinogenesis and tumor suppression. Adv Cancer Res. 66:1-40, 1995. 3.Sachs L. The control of hematopoiesis and leukemia: From basic biology to the clinic. Proc Natl Acad Sci USA. 93:4742-4749, 1996. 4.Lotem J, Sachs L. Cytokines as suppressors of apoptosis. Apoptosis. 4:187-196, 1999.

CYTOKINE SIGNALING PATHWAYS IN DEVELOPMENT AND EFFECTOR ACTION (3-7)

3

Regulation of cytokine gene expression during T cell differentiation.

Anjana Rao, Deborah C. Solymar, Dong U. Lee, and Suneet Agarwal. Harvard Medical School and The Center for Blood Research, Boston, MA 02115

Differentiation of naive T cells into mature Th2 cells is associated with appearance of a complex pattern of DNase I hypersensitive (DH) sites within the IL-4/IL-13 cytokine gene cluster. We have found that targeted deletion of an inducible DH site, VA, and the adjacent conserved DH site V (CNS-2), selectively compromises IL-4 gene transcription by differentiated Th2 cells and mast cells. In mast cells, the deletion abrogates IL-4 mRNA induction, an effect mimicked by deletion of the transcription factor NFAT1, which binds DH site VA. In T cells, the deletion impairs a process of response maturation, defined by progressive increases in IL-4 levels as Th2 differentiation proceeds. Thus the V/VA region is used by mast cells as a simple enhancer, but by Th2 cells as a means of modulating IL-4 production to the level appropriate for the immune response. The relation of CpG methylation to gene silencing is well-established, but the contribution of DNA demethylation to gene expression during cell differentiation remains unclear. We have also examined demethylation of the IL-4 locus during differentiation of naive T cells into Th1 and Th2 populations. We show that the 5' region of the IL-4 locus is hypermethylated in naive T cells and becomes specifically demethylated in Th2 cells via an IL-4/Stat6-dependent mechanism. Demethylation is not a prerequisite for chromatin remodeling. Demethylation occurs in two distinguishable phases: the early phase encompasses the transcription start site and is associated with high-level IL-4 gene transcription, while during the later phase demethylation progresses for several kilobases in the direction of gene transcription and may serve to reinforce the active chromatin structure of the IL-4 gene.

4

"Semaphorins" in the Immune Regulation

Atsushi Kumanogoh, Kazuhiro Suzuki, Chie Watanabe, Satoko Marukawa, Noriko Takegahara, Ewe Seng Ch'ng and HITOSHI KIKUTANI, Osaka University, Japan

Semaphorins have been originally identified as chemorepulsive factors which are necessary for neuronal

development. Several semaphorin molecules are also expressed in the immune system. The class IV semaphorin, CD100 (Sema4D), which is abundantly expressed on T cells, play a role in activation of B cells through its receptor, CD72. CD72 is known to function as a negative regulator for B cell responses by recruiting the protein tyrosine phosphatase, SHP-1. Interestingly, CD100 turns off negative signals of CD72 by inducing the dissociation of SHP-1 from CD72, which results in enhanced B cell responses. Production and characterization of CD100^{-/-} mice has not only confirmed a role of CD100 in B cell activation but also revealed its involvement in activation and maturation of professional antigen presenting cells. Generation of antigen-specific T cells is severely impaired in CD100^{-/-} mice. Dendritic cells (DCs) derived from CD100^{-/-} mice have a very poor ability to stimulate T cells. Recombinant soluble CD100 can significantly enhance activation and maturation of DCs. Thus, CD100 appears to be involved in generation and differentiation of antigen specific T cells by activating DCs. We have recently found that another class IV semaphorin, Sema4A, is also expressed in the immune system, particularly on DCs. T cells can express a receptor for Sema4A upon activation. Furthermore, administration of soluble Sema4A or Sema4A-specific mAb can enhance or block generation of antigen-specific T cells in immunized mice, respectively. Our findings indicate that semaphorins such as CD100 and Sema4A are essential players in regulation of immune responses.

5

Mechanisms of TRAF regulation in B lymphocyte signaling by CD40 and LMP1

Gail A. Bishop, The University of Iowa

Signal transduction by members of the tumor necrosis receptor (TNF-R) family of molecules involves association with cytoplasmic adapter proteins known as TNF-R associated factors (TRAFs). The TNF-R family member CD40, which provides important activation signals to B lymphocytes, dendritic cells, and macrophages, associates directly with TRAFs 2, 3 and 6, and indirectly with TRAF1, via its heterodimerization with TRAF2. We have found that engagement of B cell CD40 by its ligand stimulates its movement into detergent-resistant, cholesterol-enriched membrane microdomains, or rafts. CD40 ligation also recruits all the TRAFs with which it associates to these rafts, and disruption of this recruitment blocks initiation of CD40-mediated signaling cascades. Shortly after their recruitment by CD40, TRAFs 2 and 3 undergo ubiquitination and degradation, a process that requires the Zn-binding RING domain of TRAF2. We also study signaling by the EBV-encoded oncoprotein latent membrane protein 1 (LMP1); this protein is required for EBV-mediated B cell transformation. LMP1 signaling closely mimics that of CD40 in B cells, but we have shown that LMP1 signals are amplified and sustained when directly compared to CD40 signals. Although LMP1 also recruits TRAFs 2 and 3 to membrane rafts, this recruitment does not induce either their modification or degradation. Inhibition of the activity of the 26S proteasome potentiates CD40-mediated activation of c-jun kinase activity, such that the amplified signal is now similar to that delivered by LMP1 signaling. Current studies are focused upon further understanding of TRAF function and regulation in signaling mediated by the TNF-R family of molecules.

6

Decoy Receptors as a Strategy to Regulate Inflammatory Cytokines and Chemokines

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The concept of a receptor classically includes ligand recognition (usually with high affinity and specificity) and signaling. The type II IL1 receptor binds IL1b with high affinity but is not part of signaling receptor complexes. By sequestering the ligand in membrane-bound or soluble form, and by acting as a dominant negative for the accessory protein, it inhibits IL1. After definition of the IL1RII as a decoy receptor, decoy receptors have been identified among members of the IL1, TNF and IL10 family. Moreover, recent results suggest that functionally uncoupled, decoy receptors can be generated in the chemokine receptor family and that silent, non-signaling hemokine receptors can act as decoys. Therefore, decoy receptors are a general strategy to regulate inflammatory cytokines and chemokines.

7

Death domain-like modules in cell death and inflammation

Jürg Tschopp, University of Lausanne, Epalinges, Switzerland

Signaling pathways leading to an apoptotic or inflammatory response frequently involve proteins that contain the protein interaction modules called death domain (DD), death effector domain (DED), caspase-recruiting domain (CARD), and pyrin domain (PYD). For example, the cytoplasmic death domain (DD) of Fas undergoes homotypic interaction with a DD in the adaptor proteins FADD. The 'death effector domain' (DED) at the amino terminus of FADD recruits pro-caspase-8 via homotypic interaction with its two DEDs. Activated caspase-8 then initiates apoptosis by subsequent cleavage of downstream caspases (caspase-3, -6, and -7). FLIP is a caspase-8 inhibitor that modulates sensitivity toward FasL-mediated apoptosis. The long form of FLIP (FLIP(L)) exhibits an overall structural homology to caspase-8, containing two DED which interact with FADD, and an inactive caspase-like domain. We will present a novel signaling pathway that relies on proteins with PYD modules. Activation of this pathway triggers a potent pro-inflammatory response.

MECHANISMS OF SIGNAL TRANSDUCTION (8-17)

8

The interaction between gp130 and viral interleukin-6: a paradigm for the architecture of gp130-cytokine signaling assemblies

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gp130 is the shared signal-transducing receptor for a large family four-helix bundle cytokines which signal through jak/STAT cascades. A longstanding question in cytokine signaling has been the extracellular architecture of higher-order gp130 signaling complexes, as well as the structural basis for the unique promiscuous recognition properties inherent in gp130's role as a shared receptor. Kaposi's sarcoma herpes-virus (KSHV) encodes a functional homologue of human interleukin-6 (vIL6) which activates gp130, and is a growth factor in KSHV-related neoplastic diseases. We have determined the 2.4 Å crystal structure of the higher-order signaling complex between KSHV IL6 and gp130. Two vIL6/gp130 complexes are cross-linked into a tetramer which is bridged through extensive interaction between the activation (D1) domain of gp130 and a conserved region of vIL6 termed site III, which is necessary for receptor activation. The molecular mimicry of human IL-6 by the viral cytokine is achieved largely through utilization of hydrophobic amino acids to contact gp130, which substantially enhances the complementarity of the vIL6/gp130 binding interface. From the structure of this complex, biochemical and thermodynamic studies of other members of the gp130-family (e.g. CNTF, LIF) have enabled us to infer the architectures of higher-order signaling assemblies for homo- and hetero-meric gp130 and gp130/LIF-R cytokine complexes, respectively.

9

Genetic dissection of signaling in mice with targeted mutations in gp130
 B. J. Jenkins, C. Quilici, D. Grail, A. Giraud, A. Dunn and M. Ernst. Ludwig Institute for Cancer Research, Melbourne, Australia

The interleukin (IL)6 family of cytokines exhibit both functionally redundant and pleiotropic activities, a molecular basis for which is provided by the composition of their receptors which utilise the common signaling subunit, gp130. In order to identify physiological responses dependent upon the two major gp130-mediated signaling pathways, we have generated mice homozygous for "knock-in" mutations in gp130 which render the receptor incapable of activating either STAT1/3 (hereafter referred to as gp130ΔSTAT mice) or the SHP2/ras/Erk pathway (gp130Y757F mice). Gp130ΔSTAT mice display several features associated with IL6 deficiency, such as impaired immune and acute phase responses. In contrast, gp130Y757F mice, in which these responses are enhanced, partially phenocopy mice transgenic for IL6 receptor components. Similarly, both steady state platelet counts and those in response to thrombopoietic cytokines are elevated in gp130Y757F mice, whereas they are reduced in gp130ΔSTAT mice. Furthermore, both mutant mice spontaneously develop gastric lesions of differing pathologies that may be linked to altered regulation of trefoil factor family members. Notably, the gp130ΔSTAT mutation enhances ras/Erk signaling, whereas the gp130Y757F mutation enhances STAT and reduces ras/Erk activation in response to cytokines that signal via gp130. Accordingly, these two mouse models provide a unique insight into the importance of balanced activation of these two gp130-mediated signaling pathways for a wide range of biological processes, including immunity, hematopoiesis and gut homeostasis.

10

ANALYSIS OF LYMPHOID DEVELOPMENT AND IMMUNE RESPONSES IN RESCUED ADULT JAK1 DEFICIENT MICE

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Perinatal lethality of Jak1^{-/-} mice has precluded a complete analysis of the role of Jak1 in the development and function of the immune system. Recently we circumvented this early lethality by generating Jak1^{-/-}NE⁺ mice in which Jak1 is expressed under the control of the neuron specific enolase promoter in otherwise Jak1 deficient mice. IP/Western blot and RT-PCR analysis of tissue samples from the rescued adult mice demonstrated that Jak1 expression was limited to neuronal and reproductive tissues, and was not detectable in organs or cells of the immune system. Thymic from adult Jak1^{-/-}NE⁺ mice exhibited a drastic reduction in cellularity. Importantly, thymocytes displayed a significant block in maturation beyond the CD4⁺CD8⁺CD44⁺CD25⁺ stage, consistent with the inability of these cells to respond to IL-7. Although the distribution of CD4⁺ and CD8⁺-expressing thymocytes was relatively normal, those thymocytes that do mature were functionally impaired, and few T cells were found in the periphery. Examination of bone marrow from Jak1^{-/-}NE⁺ mice revealed a similar block in B cell development at the pre-pro-B to early pro-B cell stage. The near absence of DX-5⁺CD122⁺ splenocytes suggests that NK cell development was also impaired. However, cells of the myeloid lineage were over-represented and many exhibited an abnormal morphology. Moreover, macrophages from these mice were unresponsive to IFNα/β and IFNγ. Therefore, Jak1^{-/-}NE⁺ mice are severely immunocompromised due to a combined deficiency of both innate and adaptive immunity.

11

IFNβ-induction of IL-1Ra synthesis in human monocytes involves PI 3-kinase-STAT1 signaling pathway

Nevila Hyka, Marie-Thérèse Kaufmann, Danielle Burger, Jean-Michel Dayer. From the *Division of Immunology and Allergy (Hans Wilsdorf laboratory), Department of Internal Medicine, University Hospital, 1214 Geneva 14, Switzerland

IFNβ displays an anti-inflammatory property by inducing IL-1Ra without triggering synthesis of IL-1β in human monocytes (Mo). IFNβ initiates JAK-

STAT pathway which may cross-talk with components of MAP- and PI 3-kinase pathways. Since maximal activation of transcription by several STATs requires both Tyr and Ser phosphorylation, we investigated the role of MAP- (ERK1/2) and PI 3-kinases in IFNβ-induced IL-1Ra production in Mo. The PI 3-kinase inhibitor Ly294002 but not the MAP kinase inhibitor PD98052 suppresses in a dose dependent way IL-1Ra production in Mo at protein level correlating with the reduction of steady state levels of IL-1Ra mRNA. IFNβ-treatment of Mo leads to rapid Ser phosphorylation of STAT1 which is inhibited by Ly294002. Interestingly, suppression of PI 3-kinase activity in Mo stimulated by IFNβ and anti-CD11b mAb results in inhibition of IL-1Ra and upregulation of IL-1β production, suggesting that PI 3-kinase might represents a check-point signaling molecule favoring IL-1Ra synthesis. Involvement of PI 3-kinase pathway in IL-1Ra synthesis seems to be independent on differentiation state of Mo: M-CSF differentiated Mo requires activation of PI 3-kinase to synthesize IL-1Ra following IFNβ treatment. Thus, IFNβ induced IL-1Ra production in Mo by activating simultaneously components of JAK-STAT and PI 3-kinase signaling pathways.

12

Genetic Analysis of NFκB-dependent signaling pathways

Xiaoxia Li. Dept. of Immunology Cleveland Clinic Foundation

In the human embryonic kidney cell line 293, an NFκB-containing upstream region of the human IL-1 responsive gene E-selectin was used to drive the expression of proteins providing resistance to zeocin (Zeo) or sensitivity to Herpes simplex virus thymidine kinase (TK). 293-TK/Zeo cells, providing negative selection against the expression of TK in response to IL-1 were used successfully to isolate IL-1-unresponsive mutant clones. A clone lacking IRAK (IL-1 receptor-associated kinase) (IIA) has become a model system for studying the structure-function of IRAK in IL-1 signaling. IRAK is required for the IL-1-induced activation of NFκB and JNK. The goal of the current study is to understand how IRAK functions as a scaffolding protein in the activation of the intermediate proteins TAB2, TRAF6, TAK1 and TAB1. Using IRAK-deficient cells, we clearly show that IRAK, but not its kinase activity, is required for the IL-1-induced translocation of TAB2 and TRAF6 from the membrane to the cytosol, the formation of the TAK1-TAB1-TAB2-TRAF6 complex in the cytosol and the subsequent activation of TAK1. The other isolated IL-1-unresponsive clones do not lack any of the known components in IL-1 signaling pathway and are being complemented by retroviral cDNA library. In addition to the isolation of mutant cell lines, we have also developed a strategy for cloning new components of NFκB-dependent signaling pathways. The same 293-TK/Zeo cells were used to clone cDNAs whose expression leads to the constitutive activation of NFκB, by using zeocin for selection. This approach yielded one such cDNA encoding Act1, a novel NFκB activator. Act1 leads to the activation of both NFκB and JNK, suggesting that it functions before the branch point of these two pathways. Our recent studies show that the Act1-mediated activation of NFκB is through the TAK1, NIK and IKK signaling cascade.

13

Molecular Recognition between TRAF3 and TANK in Downstream TNF Signaling Pathways

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With the identification of proteins that bind directly to the cytoplasmic domains of tumor necrosis factor (TNF) receptors called TNFR-associated factors (TRAFs), an understanding of TNF-mediated activation of NF-κB and JNK signaling pathways is emerging. A downstream signal mediator TANK (TRAF-associated NF-κB activator or I-TRAF) associated with inhibition of NF-κB by CD40 and also plays an inhibitory role in activation of NFκB by TNF-α, IL-1 and LMP-1. TANK contains an amino-terminal stimulatory domain, a region required for TRAF binding and an inhibitory domain. TANK binds to TRAF1, TRAF2 and TRAF3 through interactions with the conserved TRAF-C domain in each TRAF molecule. Recognition is mediated by a 21-residue motif in TANK. Based on the results of co-immunoprecipitation assays

testing this motif, it was proposed that TANK and the cytoplasmic domain of CD40 bind to the same location on TRAFs [Cheng and Baltimore (1996) *Genes Dev.* 10:963]. In this study, we report the crystal structure of the TANK recognition motif in complex with TRAF3. We previously determined the crystal structure of a portion of the cytoplasmic domain of CD40 in complex with TRAF3 [Ni et al. (2000) *Proc. Nat. Acad. Sci. USA*]. The surprising results reveal that TANK and CD40 bind to the same binding pocket on the surface of the TRAF domain, supporting a model that TANK and CD40 (or related TNFRs such as CD30 or LMP-1) may compete for the TRAF site. Mutation of the contact residues demonstrated that the interactions seen in the crystal structure are critical for binding.

14

PATHOGENIC SIGNALING BY FLAGELLIN VIA TOLL-LIKE RECEPTOR 5 ACTIVATES THE NF- κ B AND THE PROINFLAMMATORY GENE PROGRAM.

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Salmonella infections of the gastrointestinal tract result in severe morbidity and mortality world-wide. Salmonellae are Gram-negative, pathogenic bacteria that elicit a complex genetic response in the gut mucosa, including expression of cytokines and chemokines associated with inflammation, tissue injury and shock. Previously, the Salmonella effector protein SopE, an exchange factor for the small GTPase proteins Rac and Cdc42, was thought to be the major contributing bacterial factor leading to NF- κ B activation. Using Salmonella mutants in SopE, we show that another bacterial factor is largely responsible for NF- κ B activation through the activation of the IKK α B kinase (IKK). Furthermore, this factor has been chromatographically purified and sequenced and it is the bacterial protein flagellin. Salmonella strains lacking flagellin fail to activate NF- κ B and effect expression of the proinflammatory gene program. Using Toll and IL-1 signaling component knockout cells we have identified the cellular receptor family that flagellin binds to, leading to activation of IKK and NF- κ B. Experiments using wild-type and dominant-negative Toll-like receptor (TLRs 1-9) signaling alleles have indicated that only TLR5 recognizes flagellin. Additional experiments also indicate that this recognition is enhanced by the presence of an additional bacterial protein that we have purified and sequenced. This associated bacterial protein fails to be recognized alone by the TLRs on intestinal epithelial cells.

15

IKK α and IKK β have different roles in LTbR-mediated NF- κ B activation

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The Lymphotoxin-b receptor, a member of the TNFR superfamily, functions as an essential element in the organization of lymphoid tissue and initiation of immunity for which NF- κ B signaling is involved. Treatment of embryonic fibroblasts derived from either wild type, IKK α -deficient or aly (NIK mutant) mice with agonistic anti-LTbR mAb induced equivalent NF κ B activation as measured by I κ B α phosphorylation and degradation, and p50/p65 DNA-binding. However, the response was impaired in IKK β -deficient cells. This shows that, similar to TNF-TNFR1 pathway, LTbR mediates p50-p65 activation independently of NIK and IKK α . By contrast, NIK has a role in another pathway, which involves p100 processing generating the p52 NF- κ B subunit. To address the role of IKKs in p100 processing, we used wild type, IKK α , IKK β -deficient and aly cells treated with agonistic anti-LTbR mAb. We found that LTbR-dependent p100 processing does not occur in aly and IKK α -deficient cells, but does in wild type and IKK β -deficient cells. Even the enforced expression of LTbR and NIK does not result in p100 processing in the absence of IKK α , although reintroduction of IKK α in IKK α -deficient cells restores p100 processing. In conclusion, LTbR activates two forms of NF κ B, an IKK β -dependent forming p50/p65 complex, and an IKK α /NIK dependent p100 proteolytic cleavage that generates p52, which provide for the inflammatory and developmental actions of the LTbR.

16

Cytokine-mediated activation of IKK is initiated in lipid rafts

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Exposure to pro-inflammatory cytokines (TNF α and IL-1) results in activation of signaling cascades leading to stimulation of transcription factors from the NF- κ B/Rel family. NF- κ B activity induces expression of a large number of genes that mediate immune and inflammatory responses. Stimulation of NF- κ B activity by cytokines occurs via rapid and transient activation of the I κ B kinase complex (IKK), a high molecular weight complex of 900 kD composed of two catalytic subunits, IKK α and IKK β and a regulatory subunit IKK γ /NEMO. The molecular mechanism by which IKK is activated by cytokines and the identification of the physiological activators is the subject of intense investigation. We will describe the chromatographic purification and biochemical characterization of a previously unidentified minor IKK complex which is tightly bound to the plasma membrane and strongly responsive to cytokines. All the components co-purifying with this IKK complex have been identified. In addition, cell fractionation experiments indicate the presence of the three IKK subunits in lipid rafts. Cell treatment with methyl- β -cyclodextrin dramatically reduces IKK activation by cytokines. These results suggest that cytokine-mediated activation of IKK is initiated in lipid rafts. Supported by grants from the Arthritis National Foundation and NIH (R01 AI43477).

17

ROS-dependent Activation of Interferon Regulatory Factor 3 by Lipopolysaccharide

Qanh Dang**, Lorena Navarro* and Michael David*. From **Biomedical Sciences Program, School of Medicine and *Department of Biology, University of California, San Diego, La Jolla, California 92093-0322. Interferon Regulatory Factor 3 (IRF3) is known to participate in the transcriptional induction of interferon α (IFN α) and IFN β genes, as well as a number of interferon-stimulated genes (ISGs), as a result of viral infection. Here, we show that lipopolysaccharide (LPS), a cell wall component of gram-negative bacteria, activates IRF3 with subsequent ISG induction through a p38-dependent pathway. Furthermore, we show that the induction of ISG54 by LPS, which leads to the production of reactive oxygen species (ROS), is inhibited with pretreatment of the cells with an antioxidant, N-acetylcysteine. This inhibition occurs through a glutathione-independent pathway since induction of ISG54 by LPS was still seen with cells treated with buthionine-sulfoximine, an inhibitor of glutathione synthesis. These data suggests that ROS play a regulatory role in IRF3 activation.

REGULATION OF GENE EXPRESSION (18-29)

18

TNF-alpha gene regulation in LPS or mycobacteria-stimulated monocytes is dependent upon the assembly of a unique enhanceosome and CBP/p300

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We present evidence that regulation of the human TNF-alpha gene in lipopolysaccharide (LPS)- or mycobacteria tuberculosis (Mtb)-stimulated monocytes involves the assembly of a unique enhanceosome, which is distinct from the enhanceosomes that are recruited to the TNF-alpha enhancer in T cells after virus infection or intracellular calcium flux. The TNF-alpha enhanceosome that forms in LPS or MTb stimulated monocytes is NFATp-independent and includes Sp1, ATF-2, c-jun, Egr-1, Ets/Elk and the coactivator proteins CBP/p300. Furthermore, the helical phasing relationships between transcription-factor binding sites on the TNF-alpha promoter that are required for LPS or MTb-stimulated TNF-alpha gene activation in monocytes are distinct from those required for TNF-alpha expression in activated T cells. These studies demonstrate a general mechanism by which a single gene is controlled in response to different extracellular stimuli.

19

IL-6 Regulation of the Human Methyltransferase (HDNMT) Gene in Human Erythroleukemia Cells.

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Methylation of mammalian DNA by the DNA methyltransferase enzyme (dnmt-1) at CpG dinucleotide sequences has been recognized as an important epigenetic control mechanism in regulating the expression of cellular genes. The binding of methyl-specific proteins such as MeCP1 and MeCP2 to genetic regulatory elements, represses transcription by blocking the binding of other positive acting transactivation factors. Repression of gene expression via DNA methylation has been highly indicated in tumor cell development and normal differentiation processes. The pro-inflammatory cytokine IL-6, has been associated with numerous tumor cells, of diverse tissue origins, as a proliferative and/or differentiation cytokine. Treatment of the human erythroleukemia cell line K562 with IL-6 induces the expression of megakaryocytic markers, and the silencing of certain globin genes. Here we show that IL-6 regulates the methyltransferase promoter and resulting enzyme activity of dnmt-1 in K562 cells. Extensive deletion and mutagenesis studies of the dnmt-1 promoter localized positive regulatory sites and identified the transcription factors responsible for promoter regulation as a member of the ETS family, Fli-1. The Fli-1 promoter, however, contains a Stat3 binding site and upregulation of Fli-1 could be blocked by dominant-negative Stat3 constructs. Therefore, the sequence of transcriptional events from IL-6 signal transduction proceeds from Stat3 activation of the Fli-1 promoter and the Fli-1 transcription factor then activates the dnmt-1 promoter upregulating the dnmt-1 gene and activity. We propose that this is a novel mechanism by which an inflammatory cytokine (IL-6) may permanently alter through epigenetic regulation, via DNA methylation, gene expression patterns which may effect differentiation processes as well as changes observed in tumor cell development.

20

DIRECTED CHROMATIN REMODELLING AT CYTOKINE GENE PROMOTERS FOLLOWING T CELL ACTIVATION.

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The activation of T cells by antigen stimulation leads to the production of a large number of cytokines that are important for the downstream events of the immune response. Transcription from the genes encoding interleukin-2 (IL-2) and granulocyte-macrophage colony stimulating factor (GM-CSF) is induced within hours of T cell activation leading to a transient burst of these cytokines. We have characterized the chromatin structure across the proximal promoter regions of these genes not only in T cell lines but in primary CD4⁺ T cells using a novel real time-PCR assay that we have designated CHART-PCR (CHromatin Accessibility by Real Time PCR). In unstimulated T cells, the promoters are assembled into precisely positioned nucleosomes that become disrupted or remodeled following activation. Chromatin remodeling is confined to a precise region across each promoter (300bp for IL-2 and 150bp for GM-CSF) implying that only one or two nucleosomes are disrupted. This chromatin remodeling is dependent on new protein synthesis and does not occur until 1.5-2hr following activation. There appears to be an interdependence between the assembly of transcription factor complexes and chromatin remodeling. Modification of histone protein by acetylation or phosphorylation does not, however, appear to play a major role in remodeling events at the IL-2 proximal promoter although they do affect the level of IL-2 mRNA produced. Using an in vitro recruitment assay, we find that BRG-1, a component of the SWI/SNF ATPase remodeling complex, is recruited to the GM-CSF promoter in an NF- κ B-dependent manner. In agreement with this finding, NF- κ B proteins are essential for remodeling at both the GM-CSF and IL-2 promoters. Thus, recruitment of remodeling activities to the proximal promoter regions of these cytokine genes is an important event in T cell activation.

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Expression Profiling in Lymphotoxin and Tumor Necrosis Factor Knockout Mice

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To better dissect the splenic phenotypes of knockout mice with TNF and LT deficiencies, we employed gene expression profiling approach, expecting that genes associated with phenotypic defects are actively expressed in spleens of adult wild-type (WT) mice and that differences in the expression of such genes between WT and mutant mice are due, at least in part, to changes at the level of transcription. We used hybridization to gene arrays, subtractive cloning, and Northern-blot analysis to identify genes whose expression is substantially altered in spleens of mice with single or combined TNF/LT deficiency. Several "clusters" of genes (such as lymphoid tissue specific chemokines, cell adhesion molecules, neutrophil granule proteins, and intracellular signalling molecules) were identified, suggesting defective migration of certain cell types into splenic compartments. The majority of "downregulated" genes was associated with LT deficiency rather than with TNF deficiency. Nevertheless, mRNA level of several genes were clearly diminished in TNF knockouts as well. Interestingly, the pattern of gene expression in lymph nodes is different from that observed in the spleen, suggesting distinct molecular requirements in different lymphoid tissues. Gene profiling studies failed to identify any gene whose expression was higher in spleens of LT- or TNF-deficient mice than in WT mice. Also, expression profiling helped to discover several novel genes whose specific function in the spleen remains to be established. (Funded in part by NCI Contract No. N01-CO-56000).

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Transcriptional Activation and Repression: Factor Recruitment and GRIP1 Corepressor Activity at a Collagenase-3 AP-1 Element/Tethering GRE

I. Rogatsky, K.A. Zaremba and K.R. Yamamoto. Dept. of Cellular and Molecular Pharmacology, University of California, San Francisco. Glucocorticoids repress transcription mediated by NF- κ B and AP-1, which drive the expression of cytokines, cell adhesion molecules and tissue-degrading enzymes, e.g. collagenases. We examined factor occupancy and function at an AP-1 response element, col3A, at the collagenase-3 gene in human U2OS osteosarcoma cells; col3A confers activation by phorbol esters, and repression by glucocorticoid and thyroid hormones. The subunit composition and activity of AP-1, which binds col3A, paralleled the intracellular level of cFos, which is modulated by phorbol esters and glucocorticoids. Interestingly, the collagenase-1 gene, not inducible in U2OS cells, was not bound by AP-1. The glucocorticoid receptor (GR) was tethered to col3A through an interaction with AP-1, regardless of AP-1 subunit composition, and repressed transcription. GRIP1, reportedly a coactivator for GR and the thyroid hormone receptor (TR), was recruited to col3A and potentiated repression in the presence of a GR agonist but not antagonist. GRIP1 mutants deficient in GR binding and coactivation were also defective for corepression, whereas an isolated GR-interacting region of GRIP1 was dominant-negative for repression. In contrast, repression by TR was unaffected by GRIP1. Thus, the composition of regulatory complexes, and the activities of the bound factors, are dynamic and dependent on cell and response element contexts. Cofactors such as GRIP1 may contain distinct surfaces for activation and repression that function in a context-dependent manner.

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IL-7 Controls Chromatin Accessibility for the TCR gamma V(D)J recombination via histone acetylation

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IL-7 is a key factor for the lymphoid development. Its signal is necessary for TCR gamma locus recombination. Defects in the IL-7 signal transduction pathway lead to severe immune deficiency in man and mice. We have recently reported that IL-7 controls the chromatin accessibility for RAG mediated cleavage at the TCR gamma locus. Since inhibition of histone deacetylation substituted for the IL-7 signal, we tested whether regulatory elements at the TCR gamma locus are acetylated at histones in response to IL-7 signaling by using chromatin immunoprecipitation (CHIPS). We found that the HSA, J gamma promoter and 3' enhancer were acetylated at histone 3 and 4 as measured in fetal thymocytes and RAG-/- T cell precursors. In contrast the same loci were unacetylated in thymocytes from IL-7R-/- mice. Sterile transcripts, indicating an open chromatin conformation, were suppressed in IL-7R-/- and IL-7-/-Rag-/- thymi. Within 5 hours of stimulation by IL-7, TCR gamma constant region transcripts were induced in IL-7-/-Rag-/- thymocytes. This induction correlated with an increase of histone 3 and 4 acetylation at the J gamma promoter and 3' enhancer. These results suggest that IL-7 regulates chromatin accessibility for V(D)J recombination by altering specifically histone acetylation at regulatory sites within the TCR gamma locus. These studies provide insights in the molecular mechanism by which IL-7 remodeling chromatin induces the V(D)J recombination.

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IL-1 differentially stabilizes ARE containing mRNAs

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The stability of specific mRNAs and its modulation by extracellular stimuli is determined in part by cis-acting sequences within individual mRNAs. AU-rich elements (AREs), located in the 3'UTR of early response genes, confer rapid decay to their mRNAs and can be the target of IL-1-mediated stabilization. AREs vary considerably, however, in both size and sequence context. In order to determine the relationship between ARE content, mRNA instability, and sensitivity to IL-1 mediated stabilization, human 293 cells were screened for IL-1-induced stabilization of 900 sequences present in the human AU rich mRNA containing database (ARED, Nucleic Acids Res, 2001, 29:246-54) using cDNA array analysis. RNA from untreated 293 cells was compared with that from 293 cells stimulated with IL-1 for 1 hr or stimulated with IL-1 for 1 hr followed by actinomycin D treatment for an additional 3 hrs. This allowed the identification of mRNAs induced by IL-1 that are either stable or unstable. Of 29 cDNAs that were potentially induced by IL-1, only the CXC chemokines GRO1, GRO2, GRO3 and IL-8 were also stable throughout the 3 hr actinomycin decay period. The remainder (including c-fos, c-jun, junB, and TNF induced protein A20) were induced but decayed rapidly following the termination of transcription. These findings were confirmed and extended by examination of a subset of these mRNAs using northern blot analysis. In 293 cells stimulated with TNF α both GRO1 and IL-8 mRNAs were induced but not stabilized. If, however, cells were first stimulated with TNF for 1 hr followed by actinomycin D in the presence of IL-1, both GRO1 and IL-8 mRNAs but not c-fos mRNAs were stabilized. Hence ARE sequences are also functionally heterogeneous; mRNA instability and IL-1-induced stabilization are separate properties. Supported by USPHS grant CA62220.

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Large scale analysis of AU-rich element-containing mRNA expression and turnover using cDNA microarrays

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Adenylate / Uridylate-rich elements (AREs) are present in the 3'UTRs of many mRNAs, and regulate the rapid turnover of these messages. This regulation is important in processes such as cellular growth regulation and inflammation, reflected in the types of the 57 ARE-mRNAs previously described: mainly cytokines, growth factors and some proto-oncogenes. In order to obtain a comprehensive list of ARE-mRNAs, we compiled a database by searching GenBank with a validated 13 bp ARE search pattern (Bakheet et al., 2001). We identified a large number of ARE-mRNAs (897) that encode a surprisingly wide variety of functionally diverse proteins. To investigate regulation of gene expression of all identified ARE-mRNAs in parallel we constructed a cDNA microarray containing the respective gene probes. Using the array we analyzed the inducibility of the ARE-mRNAs by lipopolysaccharide, in the presence and absence of the protein synthesis inhibitor cycloheximide. The functionality of the AREs to mediate rapid mRNA degradation was evaluated by measuring mRNA half-life using actinomycin D. In addition, we demonstrated the dependence or independence of mRNA turnover on the p38 MAP kinase signaling pathway for the entire set of ARE-mRNAs. Analyses to correlate p38 dependence with functional classes of ARE-mRNAs and with particular ARE characteristics are ongoing, as well as analyses of other signaling pathways which impact on the expression of AU-rich element containing genes.

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Mycobacterium avium Infection of Macrophages Induces Protein Binding to the 3'UTR of Nramp1 mRNA.

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Innate resistance to a number of intracellular pathogens is mediated in mice by a protein Nramp1. In addition to resistance to pathogens, Nramp1 mediates a number of pleiotropic effects. One of these effects is increased stability of a variety of mRNA species, including Nramp1. Recently we reported that Nramp1 mRNA stability was higher in *M. avium* infected RAW264.7 macrophages expressing resistant Nramp1^{Gly169} than in RAW264.7 cells expressing susceptible Nramp1^{Asp169}. The increased mRNA stability was found to result from an oxidant-generated signaling pathway that requires PKC and MAP kinases. In the present study, we examined cytoplasmic protein binding to the 3'UTR of Nramp1 mRNA. We employed a RNA mobility shift assay to identify segments in the Nramp1 3'UTR that interact with proteins. Three segments of 127, 134, and 150 bases bound proteins. Binding was induced by *M. avium* infection to higher levels in Nramp1^{Gly169} RAW264.7 cells than in Nramp1^{Asp169} cells. Competition experiments with excess unlabeled RNA showed that the three segments cross-competed with each other. Sequence alignment of the three segments also showed regions of sequence identity. UV crosslinking experiments and analysis of binding by SDS PAGE gels detected protein/RNA complexes of 30-36 kDa with each of the probes. Pretreatment with the antioxidant BHA and PKC and MAP kinase inhibitors blocked induction of Nramp1 binding proteins in Nramp1^{Gly169} RAW264.7 cells but not in Nramp1^{Asp169} RAW264.7 cells. Our studies are consistent with a model in which Nramp1 mRNA stability occurs as a result of induction of Nramp1 mRNA binding proteins by an oxidant signaling pathway involving PKC and MAP kinase. (This work was supported by grants DK-57667, AI-42901, and HL-59795 from NIH to B.S.Z. and W.P.L.).

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HUMAN IFN-gamma mRNA AUTOREGULATES ITS TRANSLATION BY STRONGLY ACTIVATING PKR

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Because interferon- γ (IFN- γ) is essential for protective immunity yet expressed transiently during a cellular immune response, translation of IFN- γ mRNA should be efficient. However, we show that human IFN- γ mRNA uses a novel mechanism to downregulate its own translation yield by over an order of magnitude, through local activation of PKR in the cell. PKR, the IFN-inducible protein kinase activated by double-stranded RNA, inhibits translation by phosphorylating the α -subunit of initiation factor eIF2. Mutations that impair the ability of IFN- γ mRNA to activate PKR strongly enhance its translation activity. These mutations map to the IFN- γ 5'-UTR. Binding and activation of PKR by IFN- γ 5'-UTR rely on distinct features because certain mutations leave affinity for PKR intact, yet severely impair kinase activation. PKR inhibitors, including 2-aminopurine, transdominant-negative mutant PKR and vaccinia E3L, correspondingly enhance translation of IFN- γ mRNA. The structure that activates PKR is located near the 5' end of IFN- γ mRNA and has been resolved by biochemical and genetic methods. The potential to form this structure is phylogenetically conserved in IFN- γ mRNA yet is not found in human IFN- α and IFN- β 5'-UTRs. We propose that in a negative feedback loop, this RNA structure acts as a sensor that adjusts translation of IFN- γ mRNA to the extracellular IFN environment. This is the first example of an mRNA that limits its own translation yield by activating PKR. This device may serve to avoid overexpression of IFN- γ protein that can lead to autoimmune diseases and toxic shock.

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Regulation of macrophage arginase I expression

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Macrophage-specific arginase I expression has been shown to regulate nitric oxide production from activated macrophages through depletion of the arginine, the substrate of iNOS. Stimulation of macrophages with LPS induces expression of both arginase and iNOS, while the Th1 cytokine IFN γ and the Th2 cytokines IL-4/IL-13 exert opposite effects on the expression of these enzymes, with the former favoring iNOS expression and the later inducing arginase. To dissect the pathways regulating expression of arginase I in macrophages we have used mice deficient in cytokines and cytokine signaling molecules. Expression of arginase was analyzed at the mRNA level using quantitative real time RT-PCR and by enzyme activity in macrophage lysates. IL-4/IL-13 induction of arginase I was strictly dependent on STAT6 signaling, whereas IL-10 required STAT3 but was unchanged in the absence of STAT6. IL-4/IL-13 induced strong expression after 4-6h hr, while stimulation with LPS resulted in high level arginase expression and activity only after overnight incubation, consistent with previous results. LPS-induced arginase was reduced in IL-10 $^{-/-}$ and STAT3-deficient macrophages, indicating that IL-10 is at least partially responsible for induction of arginase by LPS through paracrine and autocrine mechanisms. In contrast, induction of arginase by LPS was normal in STAT6 $^{-/-}$ macrophages. In STAT1 $^{-/-}$ macrophages, LPS induced very high levels of arginase I. IFN γ suppressed arginase expression following stimulation with LPS, but induced arginase in the absence of STAT1. These data suggest that the regulation of arginase expression is under complex combinatorial control through cytokine-activated JAK-Stat pathways, in contrast to the constitutive expression observed in the liver.

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Colony stimulating factor-1 differentially regulates macrophage responses to CpG DNA and LPS

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By responding to bacterial products such as lipopolysaccharide (LPS) and bacterial (CpG-containing) DNA, macrophages trigger the innate immune response and prime the acquired immune response. Although the macrophage response to LPS and CpG DNA is similar in terms of signal transduction and production of inflammatory mediators, the consequences of challenge with these stimuli in vivo are very different; LPS is highly toxic whereas CpG DNA is not. Here we show that colony stimulating factor-1 (CSF-1), which is present constitutively in vivo and is the major macrophage growth factor, differentially regulates the macrophage response to LPS and CpG DNA. CSF-1 pretreatment enhanced production of pro-inflammatory cytokines from primary bone marrow-derived macrophages (BMM) in response to LPS but suppressed pro-inflammatory cytokine production in response to CpG DNA. CSF-1 also suppressed CpG DNA-induced activation of the mitogen-activated protein kinases, ERK-1/2 and p38 in BMM. Expression of Toll-like receptor (TLR)-9, which is required for responses to CpG DNA, was dramatically suppressed in BMM in response to CSF-1. Using cDNA microarrays we have extended these findings to address the effect of CSF-1 on global gene expression in response to LPS and CpG DNA in primary macrophages.

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Null mutation of the TNF family member, APRIL, results in embryonic lethality and cardiac defects

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Members of the extensive TNF family of ligands direct a wide range of biological functions through signaling cell proliferation, survival, differentiation or apoptosis. The first identified members of these structurally related proteins were found to function in modulating the immune system. More recently discovered TNF family ligands have been found to play critical roles in organ development (EDA in hair follicle and sweat glands), homeostasis (RANKL in mammary gland and bone homeostasis), and endothelial cell proliferation and vessel formation (TWEAK). The TNF family ligand, APRIL, was discovered through its homology with other members of this family, and shares the highest homology with TWEAK, BAFF and EDA. Functionally, APRIL has been shown to promote tumor growth, but its biological role in normal tissues remains elusive. To investigate APRIL function in normal mice APRIL loss of function mutants were created by homologous recombination. Loss of APRIL function resulted in embryonic lethality at mid-gestation (10.5d \pm 11.5d pc). APRIL null embryos at 9.5d \pm 11.5d pc were runted and exhibited cardiac defects, while vascular and placental structures were formed normally. Experimental data to elucidate the mechanism of APRIL action during embryonic cardiac development will be presented.

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IL-22 IS A TIGHTLY REGULATED IL-10- LIKE MOLECULE THAT INDUCES AN ACUTE-PHASE RESPONSE AND RENAL TUBULAR BASOPHILIA.

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IL-22 is a novel cytokine that shares approximately twenty percent homology with IL-10. Murine and human IL-22 cDNAs were isolated from ConA-stimulated murine spleen cells and PHA/PMA stimulated human PBMC, respectively. Expression of the cytokine is tightly regulated, being made specifically by Th1 CD4+ T cells, as well as downstream of an inflammatory LPS signal in a variety of organs. Recent reports of others suggest that IL-22 function is also controlled/neutralized by the expression of a soluble IL-22 binding protein (IL-22BP). In contrast, the IL-22-specific and membrane-bound receptor chain (IL-22R) is expressed constitutively in a variety of organs. Either an adenovirus encoding mIL-22 or recombinant purified mIL-22 injected intravenously into C57bl/6 mice induces systemic effects including a generalized stress response, increased serum platelet levels, mild anemia, and decreased serum albumin levels. In addition, the serum amyloid A and fibrinogen levels are increased in the serum. These observations together are indicative of an acute-phase response. Microscopically, IL-22 also induces basophilia in the proximal renal tubules, a finding that is distinct from the APR.

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Identification, cloning and characterization of a novel soluble receptor which binds IL-22 and neutralizes its activity

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IL-22 is one of the recently identified novel cytokines with limited homology to IL-10. IL-22 acts through the IL-22 receptor complex consisting of the unique IL-22R1 chain and the IL-10R2 chain which also functions as the second chain of the IL-10 receptor complex. In a search for novel receptors we identified, cloned and characterized a soluble receptor designated IL-22BP (IL-22 binding protein). The *IL-22BP* gene consists of 6 exons and encodes a 231 amino acid protein with a 21 amino acid leader sequence. The secreted mature protein demonstrates 34% amino acid identity to the extracellular domain of the IL-22R1 chain. By crosslinking we demonstrated that the protein binds IL-22 and prevents binding of IL-22 to the functional cell surface IL-22 receptor complex. Moreover, IL-22BP is capable of neutralizing IL-22 activity. In the presence of the IL-22BP IL-22 was unable to induce Stat activation in IL-22-responsive human lung carcinoma A549 cells. IL-22BP also blocked induction of the SOCS3 gene expression by IL-22 in HepG2 human hepatoma cells. To further evaluate IL-22BP action we utilized hamster cells expressing a modified IL-22 receptor complex consisting of the intact IL-10R2 and the chimeric IL-22R1/γR1 receptor where the IL-22R1 intracellular domain was replaced with the IFN-γR1 intracellular domain. In these cells IL-22 activates biological activities specific for IFN-γ such as upregulation of MHC class I antigen expression. The addition of IL-22BP neutralized the ability of IL-22 to induce Stat activation and MHC class I antigen expression in these cells. Thus, the soluble receptor designated IL-22BP is a naturally occurring IL-22 antagonist which inhibits IL-22 activity by binding IL-22 and blocking its interaction with the cell surface IL-22 receptor complex.

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IL-18 Binding Protein Protects Against LPS-Induced Lethality and Prevents the Development of Fas/Fas Ligand-Mediated Models of Liver Disease in Mice.

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IL-18 binding protein (IL-18BP) is a natural IL-18 inhibitor. Human IL-18BP isoform A was produced as fusion construct with human IgG1 Fc and assessed for binding and neutralizing IL-18. IL-18BP-Fc binds human, mouse, and rat IL-18 with high affinity (KD of approximately 1 nM) in a BIAcore-based assay. In vitro, IL-18BP-Fc blocks IL-18 (100 ng/ml)-induced IFNγ production by KG1 cells (EC50=0.3 mg/ml). In mice challenged with an LD90 of LPS (15 mg/Kg), IL-18BP-Fc (5 mg/Kg) administered 10 min before LPS blocks IFNγ production and protects against lethality. IL-18BP-Fc administered 10 min before LPS blocks IFNγ production induced by LPS (5 mg/Kg) with ED50 of 0.005 mg/Kg. Furthermore, IL-18BP-Fc (5 mg/Kg) abrogates LPS (5 mg/Kg)-induced IFNγ production even when administered 6 days before LPS but shows no effect when administered 9 or 12 days before LPS. Given 10 min before LPS challenge to mice primed 12 days in advance with heat-killed *Propionibacterium acnes*, IL-18BP-Fc prevents LPS-induced liver damage and IFNγ and FasL expression. Given at the moment of priming with *P.acnes*, IL-18BP-Fc decreases *P.acnes*-induced granuloma formation, MIP-1α and MIP-2 production, and prevents sensitization to LPS. IL-18BP-Fc also prevents ConA-induced liver damage and IFNγ and FasL expression as well as liver damage induced by *Pseudomonas aeruginosa* exotoxin A or by anti-Fas agonistic Ab. In conclusion, IL-18BP can be engineered and produced in recombinant form to generate an IL-18 inhibitor, IL-18BP-Fc, endowed with remarkable in vitro and in vivo properties of binding and neutralizing IL-18.

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Bax deletion restores thymocyte development in IL-7Rα -/- mice.

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IL-7, a product of thymic epithelium, is required for normal thymocyte development. Part of the action of IL-7 has been attributed to a trophic function, in that IL-7 promotes the survival of pro-T1, T2 and T3 cells. We previously showed that, following IL-7 withdrawal, the synthesis of Bcl-2 declines sharply and the death protein Bax translocates from cytosol to mitochondria. To test the hypothesis that Bax was the principal death mediator following IL-7 withdrawal during T cell development, we crossed Bax -/- mice with IL-7Rα -/- mice and examined thymocyte development. Histologically, the IL-7Rα -/- thymus consists almost entirely of epithelium and contains virtually no lymphocytes. In contrast, the Bax -/- IL-7Rα -/- thymus was packed with thymocytes and organized into cortex and medulla, similar to wild type thymus. By flow microfluorimetry, IL-7Rα -/- thymocytes were developmentally blocked at the CD4-CD8- stage, whereas the Bax -/-IL-7Rα -/- thymus developed normal proportions of CD4+CD8+, CD4+ and CD8+ cells. However, bax deletion did not restore the total number of thymocytes, which remained intermediate between IL-7Rα -/- and WT. In vitro, Bax -/- pro-T1 cells were resistant to IL-7 withdrawal, however, pro-T2 and T3 cells remained sensitive. A candidate for the survival pathway of pro-T2 and T3 cells is PI3kinase, since inhibiting PI3 kinase killed pro-T2 and T3 cells while sparing T1 cells. These results show that Bax serves as a key counterpart to Bcl-2 in the IL-7 trophic response of pro-T1 cells and suggests that an additional death mechanism operates at the pro-T2 and T3 stages.

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Coordination of Interleukin-6 Biology by Soluble Receptors

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Cytokine receptors exist in membrane bound and soluble form. Interestingly, both forms bind their ligands with comparable affinity. While most soluble receptors are antagonists in that they compete for the ligands with their membrane counterparts, some soluble receptors are agonists. In this case, the complex of ligand and soluble receptor binds on target cells to a second receptor subunit and initiates signal transduction. Soluble receptors of the IL-6 family of cytokines are agonists. In vivo, the IL-6/sIL-6R complex stimulates several types of target cells not stimulated by IL-6 alone, since they do not express the membrane bound IL-6R. We have constructed a fusion protein consisting of IL-6 and sIL-6R fused by a polypeptide linker. The fusion protein Hyper-IL-6 is 100-1000 times more active than the separate proteins IL-6 and sIL-6R. Hematopoietic progenitor cells, neuronal cells, smooth muscle cells and stem cells do not respond to IL-6 but show a remarkable response to IL-6/sIL-6R. IL-6/sIL-6R appears to be a general switch in cellular differentiation making the fusion protein important in expansion of hematopoietic progenitor cells and embryonic stem cells. The coordination of the biological activity of IL-6 by sIL-6R and the therapeutic potential of agonistic and antagonistic strategies will be discussed [1-6]. [1] Peters et al (1998) Blood 92: 3495-3504 [2] Atreya et al (2000) Nature Med. 6: 583-588 [3] Galun et al (2000) FASEB J. 14: 1979-1987 [4] Peters et al (2000) Gastroenterology 119: 1663-1671 [5] Özbek et al (2001) Oncogene, 20: 972-979 [6] Hurst et al (2001) Immunity 14: 705-714

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Effects of IL-21 on murine T cells

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IL-21 is a recently described cytokine with homology to IL-2, IL-4, IL-15. It has been reported to modulate some NK, T and B cells responses (Parrish-Novak et al, 2000). The IL21 receptor (IL21R) is expressed by B, T, NK and NKT cells, macrophages and megakaryocytes. And, like the related receptors IL-2R β and IL-4R α it may form a functional receptor when associated with the γ -common chain (Asao et al, 2001). Here we have analyzed the effects of IL-21 on murine T cells in comparison to and in combination with IL-2 and IL-15. IL-21 enhances anti-CD3-induced proliferation of thymocytes, lymph node T cells and purified CD4⁺ and CD8⁺ T cells. Proliferation of T cells to alloantigens is also augmented. In contrast, IL-21 inhibits IL-15-mediated expansion of murine NK cells (Kasaian et al). Similar to IL-15, the effects of IL-21 on CD8⁺ T cells are particularly striking. IL-21 enhances antigen-driven proliferation of 2C TCR Tg CD8⁺ T cells. Priming of CD8⁺ T cells in the presence of IL-21 generates potent effector cells with enhanced lytic activity and increased ability to produce IFN γ . IL-21 also has effects on memory T cells and APCs. Gene expression changes in response to IL-21 are being analyzed to further evaluate this pathway. IL-21 is produced by activated CD4⁺ T cells and our data shows it acts on CD8⁺ T cells to enhance growth and CTL activity, thus IL-21 may play an important and well-regulated role in immune responses.

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Genomic Characterization of LIGHT Reveals Linkage to an Immune Response Locus on Chromosome 19p13.3 and Distinct Isoforms Generated by Alternate Splicing or Proteolysis.

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LIGHT is a member of the TNF cytokine superfamily that signals through the lymphotoxin (LT) β receptor and the herpesvirus entry mediator (HVEM).

LIGHT may function as a costimulatory factor for the activation of lymphoid cells and as a deterrent to infection by herpesvirus, which may provide significant selective pressure shaping the evolution of LIGHT. Here, we define the molecular genetics of the human LIGHT locus, revealing its close linkage to the TNF superfamily members CD27 ligand and 4-1BB ligand, and the third complement protein (C3), which positions LIGHT within the MHC paralog on chromosome 19p13.3. An alternately spliced isoform of LIGHT mRNA that encodes a transmembrane-deleted form of LIGHT is detected in activated T cells and gives rise to a non-glycosylated protein that resides in the cytosol. Furthermore, membrane LIGHT is shed from the cell surface of 293T cells. These studies reveal new mechanisms involved in regulating the physical forms and cellular compartmentalization of LIGHT that may contribute to the regulation and biological function of this cytokine. Supported in part by NIH grants AI33068, CA69381 and AI48073.

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Receptor-like properties of the 26 kDa transmembrane form of TNF

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Members of the TNF family of proteins are expressed as integral membrane proteins on lymphoid/myeloid cells. They control the proliferation, differentiation, activation and/or programmed cell death of leukocytes, progenitors (and other healthy and tumor cells). The transmembrane form of TNF and some related proteins (CD30L, CD40L, FasL) frequently interact with their receptors during cell to cell interactions inducing phenotypic changes in both cell types. This phenomenon, called reverse signaling is not well understood. According to our hypothesis the transmembrane form of TNF is a functional receptor and its signaling process can influence the expression of genes. The intracellular domain of TNF is casein kinase-phosphorylated in resting cells. Receptor-mediated clustering of TNF molecules induces dephosphorylation of the molecule by specific phosphatases and leads to Ca mobilization in the cells. preTNF is processed by TACE, a metalloproteinase. Though the enzyme cleaves both free and receptor-bound TNF molecules, the liberated, cell-associated peptides are different, as the result of the dephosphorylation of the receptor-bound molecule. We proved that the 10 kDa peptide generated by the proteolytic cleavage of preTNF carries a functional NLS sequence. The nuclear transport we observed with PE-peptide conjugate fulfilled all the criteria of a specific, selective nuclear import. In vivo experiments using an antibody against (the dephosphorylated form of) the cytoplasmic domain proved that the NLS directed the nuclear import of the 10 kDa peptide. The processed peptide was detected by immunogold technique inside the nucleus, close to nuclear pores and in association with transport vesicles. The N-terminal peptide of TNF altered the mRNA level of IL-1 β . In cells transfected with DNA construct(s) coding for N terminal peptide(s) of TNF the promoter sequence of IL-1 β showed increased activity. These observations and the fact that most members of the TNF family participate in cell fate decision events suggest that reverse signaling might also play roles in development and differentiation.

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Pre-B Cell Enhancing Factor (PBEF) Inhibits Apoptosis in Inflammatory Neutrophils (PMN)

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PBEF is a 52 kDa cytokine-like molecule, originally identified as a factor that synergizes with IL-7 to promote pre-B cell maturation. Using suppressive subtractive hybridization, we identified new transcripts for PBEF in PMN in which apoptosis had been inhibited by IL-1 β . We hypothesized that PBEF regulates PMN apoptosis during acute inflammation. **Methods:** PMN from healthy volunteers were stimulated *in vitro* with LPS (1 μ g/ml), TNF (40 ng/ml) or IL-1 β (100 pg/ml). Apoptosis was quantified cytometrically as uptake of propidium iodide, and caspase activity quantified by ELISA. PBEF transcription was inhibited with a phosphorothiate-modified antisense (AS) oligonucle-

otide, or its sense (S) control. **Results:** By 21 hours of culture, $33.4 \pm 10.8\%$ of control PMN were apoptotic ($N=10$). PBEF mRNA (Northern analysis) and protein (Western blot) were upregulated by LPS, IL-1 β or TNF, stimuli that inhibit PMN apoptosis. PBEF AS, but not S oligonucleotides abrogated the apoptotic delay: LPS $17.2 \pm 6.2\%$, LPS+S $20.1 \pm 5.6\%$, LPS+AS $40.0 \pm 10.5\%$, $p < 0.05$. Similar results were seen for TNF ($20.8 \pm 6.6\%$, AS $40.8 \pm 5.8\%$), and IL-1 ($23.0 \pm 2.2\%$, AS $38.5 \pm 1.1\%$), both $p < 0.05$. LPS decreased the activities of caspases 3 and 8 to 49.6% and 70.3% of control values respectively, an effect that could be reversed by PBEF AS. PBEF was also upregulated by LPS in monocytic U937 cells, and PBEF transcripts were detectable by RT-PCR in 4/7 critically ill septic patients, but not in healthy controls. **Conclusion:** PBEF is a cytokine produced by PMN and macrophages that plays a requisite role in the inhibition of PMN apoptosis. This novel biologic activity may justify its designation as a new interleukin. Grant Support CIHR

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Interleukin-1 homologue H4 (IL-1H4) is processed by caspase-1, binds to the IL-18 receptor but does not induce IFN- γ production

Sanjay Kumar, Charles Hanning, Michael R Burke, David Rieman, Ruth Lehr, Frank Lynch and Michael Lotze. GlaxoSmithKline Pharmaceuticals, King of Prussia, PA 19406, and Qualtek Molecular Labs, Santa Barbara, CA 93111 We have recently reported the identification of four novel members of the interleukin-1 (IL-1) family which we designated as IL-1 homologue 1-4 (IL-1H1-4) (Kumar et al., J. Biol. Chem. 275: 10308-10314, 2000). Of the four novel homologues, IL-1H4 (renamed as IL-1F7) was predicted to contain a propeptide domain and a caspase cleavage site. We now report that caspase-1 cleaves IL-1H4 at the predicted site to generate mature IL-1H4. Caspase-4 was also able to process IL-1H4 although the cleavage was very inefficient. Other caspases and Granzyme-B did not cleave IL-1H4. In a screen for a potential receptor, both pro- and mature IL-1H4 bound to the soluble IL-18 receptor α -Fc (IL-18R α -Fc) but not to the soluble IL-1R-Fc or ST2R-Fc fusion proteins. Although mature IL-1H4 bound to the IL-18R α -Fc protein with higher affinity than the pro form, affinities for both proteins were significantly lower than IL-18. Consistent with this observation, only IL-18 induced IFN- γ production from KG1a cells. In addition, IL-1H4 formed homodimers with an association constants of 6 μ M and 1 nM for pro- and mature IL-1H4, respectively. Furthermore, adenovirus-mediated expression of IL-1H4 led to processing and secretion of mature IL-1H4 in HEK 293 cells. We have also localized the expression of IL-1H4 in several colon and breast carcinoma tissues and corresponding cell lines. These data suggest that IL-1H4 may be involved in immune, cancer or inflammatory diseases.

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Combined Cytokine Stimulation of Human Naive CD4+ T Cells Results in IFN- γ Production in Absence of Hypomethylation of the IFN- γ Gene Promoter

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Stimulation of naive helper T cells with antigen or mitogen begins a process of differentiation that includes commitment to produce distinct patterns of cytokines. Th1 cells produce IFN- γ but not IL-4 while Th2 cells produce IL-4 but not IFN- γ . Cytokine production by T cells involves epigenetic changes in chromatin structure and DNA methylation. Naive CD4+ T cells have a methylated CpG dinucleotide at -52 of the transcriptional start site of the IFN- γ promoter. Differentiated Th1 cells are hypomethylated at this site, while Th2 cells remain hypermethylated. Various combinations of IL-2, IL-12, and IL-18 can induce robust production of IFN- γ *in vivo* and *in vitro*. The effect of these cytokines on that ability of naive CD4+ T cells from cord blood to differentiate into Th1 cells and produce IFN- γ was studied. Intracellular staining of the T cells shows that in 5-7 days, 30-40% of the cells produced IFN- γ in the presence of either IL-12/IL-18, IL-2/IL-12 or IL-2/IL-18. Most T cells

remained RA+ and negative for the activation marker, CD69. Furthermore, abundant mRNA for IFN- γ but little or no mRNA for IL-4 or IL-13 was detected. Cytokine ELISAs showed that cytokine treatments result in more IFN- γ production on per cell basis than mitogen activation. Single cytokine treatment did not result in IFN- γ production. Using bisulfite genomic sequencing, we found that the cytokine treatments did not alter the hypermethylation pattern at the -52 CpG site in the IFN- γ promoter. Thus, cytokine treatment can stimulate helper T cell differentiation by different mechanism(s) than mitogen activation and this activation can overcome methylation of an important regulatory site in the IFN- γ promoter.

CHEMOKINES AND CHEMOKINE RECEPTORS (42-55)

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Identification of the receptor that human LL-37 utilizes to activate human neutrophils, monocytes, and T cells

De Yang¹, Qian Chen¹, Oleg Chertov¹, Mark Anderson², Michimasa Hirata³, and Joost J. Oppenheim¹. ¹Laboratory of Molecular Immunoregulation, National Cancer Institute, NIH, Frederick, MD; ²Magainin Research Institute, Plymouth Meeting, Pennsylvania; ³Iwate Medical University, Iwate, Japan. Human LL-37 is an endogenous peptide generated from its precursor human cationic antimicrobial protein of 18-kD (hCAP18), the only identified human cathelicidin. Ever since the successful molecular cloning of its gene by three independent groups in 1995, LL-37 has been found: 1) to be expressed by neutrophils and various epithelial cells, 2) to have potent antimicrobial activity, and 3) to be able to bind and to neutralize LPS. We observed that LL-37 was chemotactic for, and induced calcium mobilization in, human monocytes, but not in monocyte-derived dendritic cells. Furthermore, modification of LL-37 by truncation or mutation resulting in increased antimicrobial and LPS-neutralizing activities caused a marked decrease in its chemotactic activity, indicating that the chemotactic activity of LL-37 could be segregated from its antimicrobial and LPS-neutralizing activities. Therefore, we hypothesized that the chemotactic activity of LL-37 might be mediated by a chemotactic receptor that is selectively expressed by monocytes, but not monocyte-derived dendritic cells. In an effort to identify this receptor, we found that the capacity of LL-37 to induce calcium mobilization in monocytes could be cross-desensitized only by a ligand specific for formyl peptide receptor-like 1 (FPRL1), but not by ligands specific for other chemotactic receptors on monocytes. Additional evidence that FPRL1 is a receptor for LL-37 is: 1) LL-37 induced chemotaxis and calcium mobilization of FPRL1-transfectant cells, but not in parental cells or cells transfected with formyl peptide receptor (FPR), a chemotactic receptor with considerable homology with FPRL1, 2) LL-37 chemoattracted neutrophils and T lymphocytes that express FPRL1, and 3) LL-37 was not chemotactic for monocyte-derived dendritic cells due to downregulation of functional FPRL1 expression by monocyte-derived dendritic cells. Taken together, we have identified the chemotactic targets of LL-37 and FPRL1 as a receptor that LL-37 utilizes to mediate its chemotactic and calcium-mobilizing effect on human leukocytes.

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CCR5-MEDIATED TYR PHOSPHORYLATION IS CRITICAL FOR RANTES-INDUCIBLE T CELL GROWTH REGULATION & POXVIRUS INFECTIVITY

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CCR5 engagement activates signaling cascades implicated in lymphocyte development, immune responses at inflammatory sites and either the clearance or transmission of infectious organisms. In earlier studies, we reported that RANTES activation of CCR5 in T cells leads to the tyrosine phosphorylation of CCR5 and multiple signaling effectors, including Jaks, Stats, p56lck and

p38MAPK. In this report we provide evidence that RANTES treatment of T cells leads to their antigen-independent growth regulation, mediated by CCR5, which is dependent on tyrosine phosphorylation events and is sensitive to inhibition by the Jak2 inhibitor, AG490 and the p38 MAPK inhibitor, SB203580. Using suppressive subtractive hybridization in conjunction with cDNA microarray technology, we have identified a panel of RANTES-inducible genes in T cells. Amongst these, Hsp90 appears to have a critical role in mediating RANTES-CCR5 regulation of T cell proliferation, through its functions in chaperone/support complexes with p56lck and signaling through the MAPK pathway. In other studies, we provide evidence that the poxviruses, myxoma and vaccinia, co-opt CCR5 for productive infection of target cells. Whereas G-protein coupled events are not essential for viral replication, CCR5-mediated tyrosine phosphorylation events, that include Jak2 phosphorylation-activation, are. Using a panel of cells that express either intact or mutant forms of CCR5, we demonstrate that specific tyrosine residues in the intracellular regions of CCR5 are critical for poxvirus infectivity. Additionally, we provide EM evidence that the intracellular fate of viral particles is determined at the level of activation of CCR5.

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Impaired hepatic granuloma formation in CCR2-deficient mice

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Granulomatous inflammation is a complicated inflammatory process in which various cytokines and chemokines are involved. In the present study we analyzed Zymosan-induced hepatic granuloma formation in CCR2-deficient mice to disclose the role of CCR2 in granuloma formation. One mg/mouse of Zymosan A was injected intravenously to CCR2(-/-) and CCR2(+/+) control mice. After the injection, the number and mean diameters of hepatic granuloma gradually increased in control mice until they peaked at 10 days. In CCR2(-/-) mice, low peak was observed at day 5, then the granuloma disappeared gradually. The numbers and the size were significantly smaller in CCR2(-/-) mice than CCR2(+/+) through 5 to 14 day period. At all time points, the ratio of polymorphonuclear cells was higher in CCR2(-/-) mice. After day 5, macrophages and epithelioid cells increased in control mice whereas increment of macrophages was unremarkable in CCR2(-/-) mice. The ratio of F4/80-positive macrophages was significantly low in CCR2(-/-) mice at day 8 and 10. Multinucleated giant cells were frequently observed in CCR2(-/-) granuloma. In CCR2(-/-) mice, serum MCP-1 level was significantly higher at day 2 and serum IFN- γ was low at day 5, 8, and 10. Through these results, CCR2/MCP-1 is considered to play an important role in hepatic granuloma formation. Impaired production of IFN- γ in CCR2(-/-) mice may relate to the appearance of multinucleated giant cells.

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Herpes simplex virus selectively induce expression of the CC chemokine RANTES/CCL5 in macrophages through a mechanism dependent on PKR and ICP0

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Recruitment of leukocytes is essential for eventual control of virus infections. Macrophages represent a leukocyte population involved in the first line of defense against many infections, among these herpes simplex virus (HSV). Through presentation of antigens to T cells and production of cytokines and chemokines macrophages also constitutes an important link between the innate and adaptive immune system. Here, we have investigated the chemokine expression profile of macrophages after HSV infection and have examined the virus-cell interactions involved. By RT-PCR and cDNA arrays, we found that HSV-1 and 2 induced expression of the CC chemokine RANTES/CCL5 in murine macrophage cell lines and peritoneal cells. The CXC chemokine BCA-1/CXCL13 was also induced. Twenty-six other chemokines tested were not affected. Accumulation of RANTES mRNA was detectable after 5 h of

infection, was sensitive to UV irradiation of the virus, and was preceded by accumulation of viral immediate-early mRNA and proteins. The viral components responsible for initiation of RANTES expression were examined with virus mutants and RAW 264.7 macrophage-like cells expressing a dominant negative mutant of the double-stranded RNA-activated protein kinase (PKR). The PKR mutant cell line displayed strongly reduced constitutive and HSV-inducible RANTES expression as compared to the control cell line. HSV-1 mutants deficient in the tegument protein VP16 or the immediate-early proteins ICP4, ICP22, or ICP27 remained fully capable of inducing RANTES expression in macrophages. By contrast the ability of ICP0-deficient HSV-1 to induce RANTES expression was compromised. Thus, HSV selectively induces expression of RANTES in macrophages through a mechanism dependent on PKR and ICP0.

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CXC chemokine redundancy ensures local neutrophil recruitment during acute inflammation

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Previous publications demonstrated that elevated systemic levels of IL-8 decrease local neutrophil recruitment. We tested whether sustained, high plasma levels of IL-8 would prevent local inflammation following inflammatory insults. Mice carrying the transgene for human IL-8 were separated on the basis of their plasma levels of IL-8 into IL-8 pos (plasma levels > 90 ng/ml) and IL-8 neg (IL-8 below detection). Presence of the IL-8 transgene did not improve survival or morbidity nor did it alter peritoneal neutrophil recruitment induced by the cecal ligation and puncture model of sepsis. In an acute lung injury model created by intratracheal injection of acid, IL-8 pos mice showed no reduction in alveolar neutrophil recruitment. There was no difference in the local recruitment of neutrophils when either thioglycollate or glycogen was injected intraperitoneally. We examined the chemotactic response to murine chemokines to test how neutrophil recruitment occurs in the setting of elevated plasma IL-8 and found that neutrophils from both IL-8 pos and neg mice respond equally well to recombinant KC or MIP-2. We measured KC and MIP-2 in the peritoneum after thioglycollate injection and demonstrated that IL-8 pos mice have significantly higher levels of the chemokines compared to the IL-8 neg mice. Antibody inhibition of KC and MIP-2 in the IL-8 pos mice significantly decreased peritoneal neutrophil recruitment in response to thioglycollate, clarifying their important role in the local neutrophil recruitment. Our data demonstrate that despite the presence of high plasma levels of IL-8, neutrophils may still be recruited to sites of local inflammation due to chemokine redundancy.

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Selective chemokine down-regulation in U-937 PMA-primed cells by an anti-inflammatory oligopeptide produced by *Entamoeba histolytica*

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The monocyte locomotion inhibitory factor (MLIF), a heat-stable anti-inflammatory pentapeptide (Met-Gln-Cys-Asn-Ser) produced by *Entamoeba histolytica* in axenic culture, in vitro inhibits the locomotion of human peripheral blood monocytes and blocks the respiratory burst in the same cells as well as in human neutrophil polymorphonuclear leucocytes. In vivo, MLIF depresses the delayed hypersensitivity skin reaction to dinitrochlorobenzene in guinea pigs and gerbils, and slows the arrival of mononuclear leucocytes in human Rebeck windows. A synthetic MLIF construct (sMLIF) displayed exactly the same selective anti-inflammatory properties. The effect(s) of MLIF on the pro / anti-inflammatory cytokine network is unknown. We explored the effect of MLIF upon chemokine regulation in resting and in PMA-primed U-937 cells in the presence sMLIF. The chemokine pattern was established by the RNase protection assay (RPA) using probes for RANTES, IP-10, MIP-1 alpha, MIP-1 beta, MCP-1, IL-8 and I-309. MLIF failed to affect the resting expression of the seven tested chemokine mRNAs but clearly down-regulated

the mRNA expression of I-309 and MIP-1 alpha in U-937 cells primed by PMA. A selective inhibition of chemokine expression may contribute in modulating the inflammatory process in autocrine and paracrine fashion, and explains some of the anti-inflammatory effects of MLIF. Supported by Coordinación de Investigación en Salud, Grant FP-0038/1013 IMSS, México.

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Structural and Functional Characterization of Human CXCR4 as a Chemokine Receptor and HIV-1 Co-receptor by Mutagenesis and Molecular Modeling Studies

Naiming Zhou^{1, 2}, Zhaowen Luo¹, Jiansong Luo¹, Dongxiang Liu¹, James W. Hall¹, Roger J. Pomerantz², and Ziwei Huang^{1, 3}. 1 - Kimmel Cancer Center 2 - The Dorrance H. Hamilton Laboratories, Center for Human Virology, Division of Infectious Diseases, Department of Medicine Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania 19107 3 - Department of Biochemistry, School of Molecular and Cellular Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801 The human CXC chemokine receptor 4 (CXCR4) is a receptor for the chemokine stromal cell-derived factor (SDF-1) and a co-receptor for the entry of specific strains of human immunodeficiency virus type I (HIV-1). CXCR4 is also recognized by an antagonistic chemokine, the viral macrophage inflammatory protein II (vMIP-II) encoded by human herpesvirus 8. SDF-1 or vMIP-II binding to CXCR4 can inhibit HIV-1 entry via this co-receptor. An approach combining protein structural modeling and site-directed mutagenesis was used to probe the structure-function relationship of CXCR4, and interactions with its ligands SDF-1 and vMIP-II and HIV-1 envelope protein gp120. Hypothetical three-dimensional structures were proposed by molecular modeling studies of the CXCR4-SDF-1 complex, which rationalize extensive biological information on the role of CXCR4 in its interactions with HIV-1 envelope protein gp120. With site-directed mutagenesis, we have identified that the amino acid residues Asp (D20A) and Tyr (Y21A) in the amino-terminal (NT) domain and the residue Glu (E268A) in extracellular loop 3 (ECL3) are involved in ligand binding, whereas the mutation Y190A in extracellular loop 2 (ECL2) impairs the signaling mediated by SDF-1. As the HIV-1 co-receptor, we found that the NT domain, ECL2, and ECL3 of CXCR4 are involved in HIV-1 entry. These structural and mutational studies provide valuable information regarding the structural basis for CXCR4 activity in chemokine binding and HIV-1 viral entry, and could guide the design of novel targeted inhibitors.

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CXCL10 INHIBITS CXCL1-INDUCED SIGNALING BY INTERRUPTING CXCL1 BINDING ACTIVITY TO CXCR2 RECEPTOR

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The CXC subfamily of chemokines plays an important role in diverse processes, including inflammation, wound healing, growth regulation, angiogenesis, and tumorigenesis. We have previously demonstrated that the expression of CXCL1, enhances M-Ras expression and activity, which results in the activation of NF- κ B through MEKK1-MEK3/6-p38 MAP kinase cascade. The activation of NF- κ B is required for protection from M-Ras-induced apoptosis. CXCL10 is chemotactic for activated T cells and inhibits the angiogenic functions of the CXCL1 and CXCL8 chemokines as well as bFGF and VEGF. However, the underlying mechanisms for the angiostatic properties of CXCL10 are poorly understood. We show here that CXCL1 induces the activation of PAK1 and ERK1/2 in CXCR2-expressing HEK293 cells. PAK1 activation is required for CXCL1-induced chemotaxis. However, ERK1/2 activation is not involved in this ligand-induced chemotaxis and is not a downstream target for PAK1, based on the observation that the expression of the dominant negative ERK does not block CXCL1-induced chemotaxis and the dominant negative PAK1 does not block CXCL1-induced ERK activation. CXCL10 inhibits CXCL1-induced PAK1 and ERK activation as well as the CXCL1-induced chemotaxis. Moreover, the maximal binding activity of

CXCL1 to its receptor requires the presence of cell surface proteoglycans, based on the observation that CXCL1 binding to CXCR2-expressing cells is inhibited by heparan sulfate and is partially blocked when the cells are treated with heparinase. Since CXCL1 activation of PAK1 and ERK are inhibited when cells are treated with heparinase or with CXCL10, we postulate that proteoglycan presentation of CXCL1 to CXCR2 is important for maximal signaling and the binding of CXCL1 to proteoglycan is competed by CXCL10

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EXPRESSION OF GROWTH-RELATED ONCOGENE (GRO)-ALPHA BY HUMAN EOSINOPHILIC GRANULOCYTES

Terese Persson, Pia Andersson, Anders Bjartell and Arne Egesten. Departments of Medical Microbiology (T.P, P.A and A.E) and Urology (A.B), Lund University, Malmö University Hospital, Malmö, Sweden Eosinophils are seen in concurrence with neutrophils and mast-cells at sites of inflammation, for example in asthma and ulcerative colitis. In the present study, we investigated the expression of the neutrophil- and mast-cell activating CXC-chemokine growth-related oncogene (GRO)- α by human eosinophils. Freshly isolated eosinophils expressed GRO- α as detected by *in situ*-hybridization and immunocytochemistry, containing approximately 20 pg GRO- α / 10^6 cells. During prolonged incubation of eosinophils, a strong increase in GRO- α of the supernatants was seen with time. After addition of the protein synthesis inhibitors actinomycin D or cycloheximide, GRO- α could no longer be detected in the medium while the intracellular GRO- α content remained unchanged, showing that eosinophil GRO- α release is dependent on *de novo* synthesis. Varying levels of GRO- α expression was seen in eosinophils obtained from different donors, but could not be attributed to atopic or non-atopic constitution. Addition of the eosinophil-activating cytokine IL-5 to eosinophils from low-expressing donors increased GRO- α expression. The present study suggests that eosinophils can have proinflammatory effects through expression of GRO- α contributing to recruitment and activation of CXC-receptor 2 (CXCR2) bearing cells, such as neutrophils and mast cells at sites of inflammation.

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Detection of a Novel Component of CCR5 Ligand Induced Trafficking

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We previously characterized a number of naturally occurring CCR5 variants for their role in HIV 1 infection and observed that variants in the first transmembrane domain, first intracellular loop and second transmembrane domain were permissive for HIV-1 infection, but exhibited altered responses to CCL5 (RANTES). Receptor variants in these domains responded chemotactically to CCL5 when expressed in a human embryonic kidney cell line (HEK/293), but instead of the classic bell shaped response curve, these variants showed a saturation response to CCL5 and could not be desensitized by CCL5. We therefore, investigated ligand induced receptor internalization and receptor associated proteins. We observed that CCL5 induced rapid internalization of the I42F CCR5 variant within 1 minute while 25 minutes were required for CCL5-induced internalization of the wild type CCR5. Additionally, we detected that chaperonin containing t-complex polypeptide 1 (TCP-1) co-precipitated with the I42F variant at 1 minute, but with the wild type CCR5 only by 25 minutes. TCP-1 is a cytoplasmic protein that modulates cytoskeletal protein assembly. These studies reveal that changes in the components of the first transmembrane domain of CCR5 regulate the kinetics of TCP-1 association with CCR5. Furthermore, these findings identify TCP-1 as a novel component of ligand induced CCR5 receptor trafficking and we hypothesize that this pathway maybe used by other Gi protein coupled receptors.

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Truncation of N-terminal amino acid residues of leukotactin-1 increases agonistic potency on CCR1 and CCR3

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Leukotactin-1 (Lkn-1) is a human CC chemokine that binds to both CC chemokine receptor (CCR)1 and CCR3. Structurally, Lkn-1 is distinct from other human CC chemokines in that it has long amino acid residues preceding the first cysteine at the N-terminus, and contains an extra two cysteines. N-terminal amino acids, 1-31, of Lkn-1 were deleted serially and the effects of each deletion were investigated in CCR1 or CCR3 expressing cells. In CCR1 expressing cells, deletion up to 20 amino acids ($\Delta 20$) did not change the agonistic potency significantly. Deletion of 24 amino acids ($\Delta 24$), however, increased agonistic potency at least 50 fold in terms of calcium flux. Deletion of 28 amino acids ($\Delta 28$) also increased agonistic potency to the same level shown by $\Delta 24$. Deletion of one more amino acid ($\Delta 29$), however, abolished the agonistic activity almost completely. Loss of agonistic activity of $\Delta 29$ was due to inability to bind to CCR1. In CCR3 expressing cells, $\Delta 24$ was the only form of Lkn-1 which revealed increased agonistic potency. Our results indicate that at least 3 amino acid residues preceding the first cysteine at the N-terminus are essential for the biological activity of Lkn-1, and further suggest that N-terminal processing is a potential mechanism for the regulation of biological activities.

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Identification of cis-acting DNA elements required for PMA-induced transcription of the Human Leukotactin-1 and Ck β 8 genes

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Leukotactin-1 (Lkn-1) and Ck β 8 belong to a family of CC chemokines, which are known to act as chemoattractant for monocytes, basophils, eosinophils, T lymphocytes, NK cells, and dendritic cells. The CC chemokine gene family were clustered on chromosome 17, region q12. It has been reported that Lkn-1 and Ck β 8 are tissue-specifically expressed and induced by treatment with IL-4 or PMA plus LPS. However, little is known about transcriptional regulation of Lkn-1 and Ck β 8 genes. In the present study, we have identified a distal DNA sequence in the 5'-flanking sequences of Lkn-1 gene and a proximal DNA sequences in the 5'-flanking sequences of human Ck β 8 gene for induction of transcription of the genes by PMA. The PMA-responsive DNA sequences of Lkn-1 is located between -2130 and +1860 bp upstream from the translation initiation codon. The PMA-responsive DNA sequences of Ck β 8 gene is located within +1.0kb upstream from the translation initiation codon. A putative Sp1 binding site and p53 binding site were contained in the regions, respectively. Specific binding of Sp1 and p53 to the sites were detected by gel mobility shift assays.

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LEPTIN INDUCES THE SECRETION OF IP-10, BUT NOT RANTES, ON HUMAN MONOCYTIC CELLS

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Since it is known that leptin induces the production of IL-1Ra in human monocytes (Mo) (*J Clin Endocrinol Metab* 86: 783, 2001), we have investigated whether leptin would modulate other factors expressed in Mo. We found that IP-10 mRNA was strongly induced on the human monocytic cell-line THP-1 treated with leptin, while RANTES mRNA - constitutively expressed - was not affected. Leptin had no effect on the secretion of IL- α , MMP-1 or TIMP-1, confirming the selectivity of the effect of leptin. Low concentrations

of leptin (10 nM) induced a 2-fold increase in IP-10 secretion, and at 500 nM its effect was maximal, resulting in 45-fold induction. In contrast, the secretion of RANTES was unaffected even at the highest concentration of leptin. In freshly isolated human Mo leptin induced the 5-fold production of IP-10. In conclusion, physiological concentrations of leptin result in the strong and selective induction of IP-10 on human monocytic cells. Since high levels of leptin are a constant feature of obesity, which in turn predisposes to cardiovascular disease, it is tempting to speculate that the proinflammatory effects of leptin may contribute - through the migration of cells mediated by specific chemokines - to the progression of atherosclerotic lesions.

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Macrophage inflammatory protein (MIP)-3 α expression is increased in human inflammatory bowel disease

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MIP-3 α , the ligand of CCR6, has recently been proposed the key chemokine attracting Langerin⁺ Langerhans cell-like dendritic cells (DCs) to inflamed epithelial cell surfaces. Inflammatory bowel disease (IBD), constituting Crohn's disease (CD) and ulcerative colitis (UC), are characterized by infiltration of Th1 and Th2 lymphocytes, respectively, in the lamina propria of the gut. In the current study, we asked whether MIP-3 α expression is altered in IBD. MIP-3 α mRNA levels in colonic biopsies of IBD patients and healthy controls were determined by real-time PCR analysis. Whole tissue cultures of colonic biopsies were analyzed for MIP-3 α secretion by ELISA. MIP-3 α and subsets of mucosal DCs were identified by immunohistochemistry. MIP-3 α mRNA levels were significantly elevated in CD compared to controls. MIP-3 α mRNA levels varied widely in UC patients, and were not statistically different from controls. In vitro MIP-3 α secretion from colonic biopsies were elevated in CD compared to controls. In UC, MIP-3 α secretion was only elevated in lesional specimen. Enhanced MIP-3 α was localized to inflamed epithelium. Langerin⁺ DCs were virtually absent from IBD mucosa. Few CD1a⁺ and DC-LAMP⁺ DCs were identified. We demonstrate upregulation of MIP-3 α in IBD. Despite markedly elevated MIP-3 α levels, Langerin⁺ DC recruitment to inflamed surfaces was not increased in IBD. Enhanced MIP-3 α expression in IBD might be related to the attraction of regulatory CD4⁺CD25⁺ T lymphocytes expressing CCR6 and the gut-homing $\alpha_4\beta_7$ integrin.

PROINFLAMMATORY SIGNALING PATHWAYS (56-62)

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DIFFERENTIAL REGULATION OF IP-10 EXPRESSION IN HUMAN AIRWAY SMOOTH MUSCLE BY INF γ AND TNF α : ROLE OF PROTEIN KINASE C α

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INF- γ -inducible protein-10 (IP-10) is a CXC chemokine that binds with specificity to CXCR3 and is dramatically upregulated by IFN γ . While IFN γ induces IP-10 expression in all cells studied, some cells express IP-10 in response to TNF α . For example, TNF α -induces IP-10 in canine endothelial cells (Frangogiannis 2000 Cell Tissue Res.), but not in PBMCs (Gasparini 1999 J. Immunol.). Thus differential regulation of IP-10 based on cell type may exist. IP-10 is made by many cell types, including granulocytes and activated bronchial epithelial cells (Sauty 1999 J. Immunol). Expression of IP-10 by these cells, combined with the presence of CXCR3⁺ T lymphocytes in the lungs of patients with chronic obstructive pulmonary disease (COPD), implicate the chemokine/receptor pair in the pathogenesis of COPD. While it is known that the bronchial epithelia participate in the inflammatory response by producing IP-10, it is not known whether human airway smooth muscle (hASM) expresses IP-10. In this study, we demonstrate expression of IP-10 protein and mRNA in hASM induced by either IFN γ or TNF α , and an

increased effect when the two are combined. This is significant, since high levels of IFN γ and TNF α are detected in the lungs of patients with COPD. In addition, IFN γ -induced IP-10 expression, vs. TNF α [1537], is differentially inhibited by the Th2 cytokines, IL-4, IL-5 and IL-10. Finally, we used inhibitors of signalling molecules to identify a role for PKC ϵ in the regulation of IP-10 expression by IFN γ and TNF α . These data suggest hASM participation in the pulmonary inflammatory response by expressing IP-10.

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REGULATION OF IL-8 PRODUCTION AFTER HUMAN KERATINOCYTES UVB-IRRADIATION

GRANDJEAN-LAQUERRIERE A.J., GANGLOFF S.C.1, LE NAOUR R.1, TRENTSAUX C.2, HORNEBECK W.3, GUENOUNOU M.1. IFR 53 Biomolécules, INSERM, CNRS, 1 avenue du Maréchal Juin, 51100 Reims, France : 1Laboratoire d'Immunologie (EA2070), 2Laboratoire de Biologie Moléculaire, (FRE CNRS 2141), 3Laboratoire de Biochimie Médicale et Biologie Moléculaire, (FRE CNRS 6021) . IL-8 is one of the most extensively studied chemokine. And even so it is considered as the primary regulatory molecule of acute inflammation states, little is known about its gene-regulatory mechanisms underlying UVB radiation-induced production by human keratinocytes. To characterize these mechanisms, the impact of NF- κ B and AP-1 nuclear transcription factors as well as the protein kinase (PKA, PKC) pathways were analyzed. IL-8 UVB-induced overexpression was accompanied by NF- κ B and AP-1 transcription factors activation as assessed by EMSA. PMA, known to be a strong PKC activator, had the same effects. Furthermore, treatment by Calphostin C, a PKC inhibitor, reduced the IL-8 UVB induced production by the keratinocytes but D609, a PLC inhibitor that operates upstream PKC, did not. In opposition, the PKA up-regulation induced by increasing cAMP levels due to external compounds (dibutyl cAMP, PGE2 and CT) had no effect on the IL-8 production. But irradiated cells treatment with PKA specific inhibitors (H89 and PKAi) leads to a significant inhibition of IL-8 release. Taken together, our data indicate that the IL-8 production in UVB-irradiated human keratinocytes is dependent on NF- κ B and AP-1 transcription factors activation but also to a certain extent on the PKC and PKA pathways.

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TNF Receptor Associated Factor (TRAF) 1 is a negative regulator of TNFR2 signaling: Enhanced TNF signaling in TRAF 1 deficient mice
Erdyni N. Tsitsikov*, Dhafer Laouni*, Ian F. Dunn*, Tatyana N. Sannikova*, Laurie Davidson#, Fred W. Alt# and Raif S. Geha *. *Division of Immunology and #Howard Hughes Medical Institute, Children's Hospital and Department of Pediatrics, Harvard Medical School, Boston MA. TRAF1 is a unique TRAF protein, which lacks a ring finger domain and is predominantly expressed in activated lymphocytes. To elucidate the function of TRAF1, we generated TRAF1 deficient mice. While TRAF1 $^{-/-}$ mice are viable and have normal lymphocyte development, TRAF1 $^{-/-}$ T cells exhibit stronger than wild type (WT) T cells proliferation to anti-CD3 mAb, which persisted in the presence of co-stimulation with anti-CD28 antibodies or upon addition of IL-2. These results suggest that TRAF1 normally inhibits TCR/CD3 mediated activation by interfering with other than CD28 or IL-2 signaling pathways, which may include the TRAF1-associated TNFR family members, including TNFR2, CD30, OX40, CD27, 4-1BB, HVEM/ATAR and AITR. In vivo examination of peripheral T cell clonal expansion and deletion following TCR signaling after injection of the superantigen SEB revealed no differences between TRAF1 $^{-/-}$ and WT mice. Interestingly, TNF caused marked proliferation of pre-activated T cells from TRAF1 $^{-/-}$ mice compared to WT T cells. In agreement with the selective expression of TNFR2 on activated T cells, the response of TRAF1 $^{-/-}$ T cells to TNF was completely abrogated by blocking antibodies to TNFR2, but not by antibodies to TNFR1, indicating that TRAF1 inhibits activation signaling via TNFR2. Furthermore, activation of the NF- κ B and AP-1 pathways in response to TNF was enhanced in TRAF1 $^{-/-}$ T cells compared to WT T cells. We used a model of TNF-induced skin necrosis to examine the effect of TRAF1 deficiency on TNF signaling in vivo. We have

found that lymphocytes play an important role in this in vivo effect of TNF, because RAG-2 $^{-/-}$ mice were resistant to TNF-mediated skin necrosis. TRAF1 $^{-/-}$ mice were found more susceptible to TNF-induced skin necrosis than WT mice, suggesting that TRAF1 normally protects skin from lymphocyte-mediated TNF-induced necrosis. Taken together these results suggest that TRAF1 is a negative regulator of TNF signaling through TNFR2 in activated T cells.

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Direct effect of proinflammatory cytokines on human myocardial contractile function if ICE dependent and is mediated by NO

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Myocardial contractile dysfunction following an ischemia/reperfusion (I/R) injury is mediated by the production and release of proinflammatory cytokines. We have previously demonstrated that blockade of the IL-1 receptor, TNF α , and IL-18 attenuates post-I/R myocardial dysfunction. To assess the role of IL-18 in the pathogenesis of cytokine-induced contractile dysfunction, we hypothesized that IL-18 was a critical mediator of proinflammatory contractile depression. We measured myocardial contractile force in superfused human atrial trabeculae in the presence of oxygen and glucose; the addition of TNF α suppressed contractile force. Using IL-18 binding protein, TNF-induced suppression of contractile force was attenuated, suggesting that endogenous IL-18 mediates TNF effects on the myocardium. Using inhibition of caspase-1, (ICE inhibitor YVAD) TNF-induced contractile depression was also abrogated, suggesting that TNF activates ICE in cardiac macrophages (M ϕ) with subsequent processing of endogenous IL-18. Not unexpectedly, exogenous IL-18 suppressed contractile force. Since nitric oxide (NO) has been implicated in the pathogenesis of cytokine-mediated contractile dysfunction, we observed that NO inhibition reversed IL-18-induced contractile dysfunction. In murine primary M ϕ , TNF-induced NO production was suppressed by ICE inhibition. We conclude that TNF α -induced myocardial contractile depression is IL-18 dependent via activation of ICE. In addition, NO appears to be the final common mediator of proinflammatory cytokine-induced myocardial contractile dysfunction.

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Differential regulation of Toll-like receptor mRNAs by microbes, their products & cytokines

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Toll-like receptors (TLR) mediate patterning and cellular adhesion in insects as well as immune responses in insects and mammals. TLRs have extracellular leucine-rich repeat domains and an intracellular signaling domain (TIR) that shares homology with the IL-1 receptor and plant Resistance genes. Human TLR1-10 and RP105 (which lacks a TIR) have been cloned as have mouse orthologues. Only five have known functions in recognition of LPS, lipoproteins, flagellin and unmethylated CpG DNA. To identify potential sites of action, we used quantitative real-time RT-PCR to measure expression of mRNAs encoding TLRs and several proteins important in TLR functions (MD1, MD2, CD14, MyD88). Most tissues expressed at least one TLR and several expressed all (spleen, peripheral blood leukocytes). Analysis of TLR expression in CD4 $^{+}$, CD8 $^{+}$, CD19 $^{+}$, monocytes and granulocytes indicates that professional phagocytes express the greatest variety of TLR mRNAs although several TLRs are restricted to B-cells suggesting additional roles for TLRs in adaptive immunity. Monocyte-like THP1 cells alter TLR mRNA levels in response to phorbol esters, LPS, bacterial lipoproteins, live bacteria, and cytokines. Addition of *E.coli* to human blood *ex vivo* caused distinct changes in TLR expression in monocytes and granulocytes. Infection of mice with *S.typhimurium* also resulted in changes in TLR mRNA levels in several tissues. Alteration in TLR expression during infection and inflammation likely serves an important host defense role.

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Injury augments Toll-like receptor responses independently of TLR4

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INTRODUCTION: Major injury causes dysregulated immune function, characterized in the clinical setting by impaired resistance to infection. The interleukin-1 (IL-1) family of cytokines and receptors act as early mediators of immune and inflammatory reactions. Mammalian Toll-like receptors (TLRs), homologues of *Drosophila* Toll, are structurally similar to IL-1 receptors and mediate responses to bacterial components via a common signaling pathway. We used a mouse model of thermal injury to examine the effect of injury on TLR-mediated responses in wild-type and TLR4-deficient mice. **METHODS:** C57BL/10SnJ (wild type, WT) and C57BL/10SnCr (TLR4-deficient) mice were anesthetized, placed in a mould to expose 25 total body surface area and immersed in 90C (injury group) or isothermic (sham injury group) water. 3 hours or 7 days later, cell suspensions were made from spleens and draining lymph nodes and stimulated in culture with a variety of TLR2-associated (peptidoglycan(PGN), lipoteichoic acid(LTA)) and TLR4-associated (lipid A, lipopolysaccharide(LPS)) ligands. To ascertain the source of / interaction of cell subsets required for cytokine production within these preparations, total splenocyte cultures were selectively depleted of T-cells or APCs using immunomagnetic beads. Production of IL1, IL6 and TNF was measured by ELISA after 48hrs culture. **RESULTS:** There was no significant difference in cytokine production between sham-injured and injured groups at 3 hours. Injury substantially augmented IL1, IL6 and TNF production at 7 days in wild type lymph nodes and spleen in response to both TLR2 and TLR4 ligands. TLR4-deficient cells were fully responsive to PGN and hyporesponsive to LTA and LPS; the same pattern of injury-enhanced response was nevertheless observed. T-cell depletion reduced overall cytokine production and diminished the injury-induced augmentation. **CONCLUSIONS:** Injury causes enhanced TLR-mediated reactivity. This enhancement is observed in TLR4-deficient mice and is therefore independent of TLR4. T-cells are required for a full TLR-mediated response and contribute substantially to the augmented response seen after injury. β α β α %

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Mal (MyD88 Adapter-like) is required for Toll-like receptor-4 signal transduction

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The recognition of LPS by the innate immune system involves Toll-like receptor-4 (TLR-4), a member of the IL-1/TLR receptor superfamily. Similar to other TLRs, TLR-4 has a Toll/IL-1 receptor (TIR) domain which is responsible for signal transduction. MyD88 is a cytosolic protein which also has a TIR domain. During signalling it is recruited to receptor TIR domains, acting as an adapter. Our understanding of how TLR-4 signals is incomplete however, since cells from MyD88 - deficient mice still display NF- κ B activation in response to LPS, although the response is impaired. Here, we describe Mal (MyD88 adapter-like), which joins MyD88 as another adapter protein with a TIR domain. Unlike MyD88 it lacks a death domain. Mal activates NF- κ B, JNK and p42/p44 MAP kinase. It can form homodimers or can heterodimerise with MyD88. Mal signalling requires IRAK-2 but not IRAK, distinguishing it from MyD88 which requires both IRAKs. A dominant negative form of Mal inhibits signalling by TLR-4 but not IL-1, IL-18 or TLR-2, and Mal associates with TLR-4 but not TLR-2. Mal is therefore a novel adapter, specifically involved in TLR-4 signal transduction.

NF-KAPPA B (63-65)

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Prion protein activation of the NF κ B signalling pathway in human monocyte derived dendritic cells

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Specific peptides derived from the prion protein (PrP) sequence have been examined for their potential cytotoxic and neurotoxic effects when used in vitro or in-vivo. One such peptide, the PrP106-126 fragment has been shown to mimic the pathologic isoform of prion protein and to induce a cytotoxic effect in neuronal cells. To characterize the role of follicular dendritic cells in this process and to determine the potential mechanism whereby this cytotoxicity can occur, we derived dendritic cells from elutriated human monocytes and determined the ability of PrP106-126 to activate specific signalling pathways in these cells. Dendritic cells exposed to PrP106-126 underwent an activation of intrinsic NF κ B, as demonstrated by electrophoretic mobility shift assays. Activation of NF κ B was observed within 30 minutes of exposure, with a maximum activation seen at 120 minutes. Because NF κ B is involved in the regulation of inflammatory mediators, we analyzed inflammatory cytokine gene expression in dendritic cells responding to PrP106-126 by RNase protection assays. The data demonstrate a marked induction of mRNA for IL-1 α , IL-6, and TNF α in a time-dependent manner. This increased expression of mRNA was reflected in the concomitant accumulation of the protein, as demonstrated by ELISA analysis of the culture supernatant. These data demonstrate the ability of prion protein fragments to induce inflammatory cytokines through the NF κ B signalling pathway and suggest a potential mechanism by which prion proteins may exert their neuro- and cytotoxicity.

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Both IKK α and IKK β are required to phosphorylate and activate the p65 subunit of NF κ B in response to cytokine-stimulated Phosphatidylinositol-3-Kinase and Akt.

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Previous work from our laboratory and others has demonstrated a critical role of phosphatidylinositol-3-kinase (PI3K) and the serine/threonine kinase Akt in phosphorylating and activating p65 in response to interleukin-1 (IL-1) and tumor necrosis factor (TNF). Studies of mice lacking specific IKK subunits have demonstrated that IKK β but not IKK α , is largely responsible for cytokine-induced I κ B degradation and NF κ B nuclear translocation. However, IKK α -null mouse embryo fibroblasts (MEFs) are deficient in induction of several NF κ B-dependent mRNAs in response to IL-1 and TNF. Therefore, we investigated the potential roles of IKK α and IKK β in activating the p65 subunit. Both IKK α and IKK β null MEFs are deficient in NF κ B-dependent promoter activation and gene induction following stimulation with IL-1 and TNF. However, the IKK α -null MEFs are not defective in the cytokine-stimulated I κ B α degradation or NF κ B nuclear translocation that is observed with IKK β -null MEFs. In contrast, the IKK complex from both IKK α - and IKK β -null MEFs is deficient in the PI3K-mediated phosphorylation of the transactivation domain of p65 NF κ B in response to IL-1 and TNF. Neither constitutively activated PI3K nor Akt is able to potentiate cytokine-stimulated activation of NF κ B in either the IKK α - or IKK β -null MEFs. Only reconstitution with IKK α , and not IKK β , restores cytokine-stimulated NF κ B-dependent promoter activation and endogenous gene induction in the IKK α -null MEFs, indicating a separate essential function of IKK α . Collectively these data indicate that in contrast to IKK β , which is required for both NF κ B liberation and p65 NF κ B phosphorylation, IKK α plays a novel essential role in the PI3K/Akt-mediated phosphorylation and activation of the p65 subunit of NF κ B by IL-1 and TNF.

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The synergistic effect of IL-1 β on induction of C-reactive protein (CRP) by IL-6 can be reproduced by overexpressed NF- κ B

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The acute phase protein CRP is modestly induced by IL-6 in the human hepatoma cell line Hep3B. IL-1 alone has no effect on CRP expression, but markedly enhances the effect of IL-6. In studies of the proximal CRP promoter, we found that STAT3 and C/EBP β mediate IL-6-induced transcription and that C/EBP β and rel p50, but not p65, can act synergistically to induce CRP transcription. The current studies were undertaken to determine whether these transcription factors regulate expression of the endogenous CRP gene in a comparable manner. Overexpression of p50 and p65 in Hep3B cells induced CRP mRNA accumulation, assessed by northern blot analysis, in a dose-dependent manner. The heterodimer p50/p65 was more effective than the homodimers p50/p50 or p65/p65. Overexpression of C/EBP β or STAT3 induced CRP expression minimally; the response to their combination was additive. Co-overexpression of p50/p65 with C/EBP β or STAT3 synergistically enhanced endogenous CRP expression. Maximal CRP expression was observed in cells co-overexpressing p50/p65, C/EBP β and STAT3 and was comparable to that observed in IL-1 β -treated cells co-overexpressing C/EBP β and STAT3. Data generated by the Human Genome Project permitted us to identify a number of potential κ B sites in the first 4,000 bases of the CRP promoter. We tested 13 of these by EMSA analysis for the ability to bind the nuclear p50/p65 heterodimer activated by IL-1 β in Hep 3B cells. Only one of them, centered at -2652, was found to do so. Taken together, our findings indicate that classical NF- κ B activation participates in endogenous CRP induction and support the view that the synergistic action of IL-1 β on IL-6-induced CRP expression may be mediated through NF- κ B activation.

MAPK, STRESS PATHWAY (66-68)

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A MEK Inhibitor, PD98059 Enhances IL-1-induced NF- κ B Activation by the Enhance And Sustained Degradation of I κ B α

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IL-1 mediates numerous host responses, through rapid activation of NF- κ B, but signal pathways leading to the NF- κ B activation appear to be complicated and multiplex. We propose a novel regulatory system for NF- κ B activation by the extracellular signal-related kinase (ERK) pathway. In a human glioma cell line, T98G, IL-1-induced NF- κ B activation was markedly augmented by the pretreatment of a specific MEK inhibitor, PD98059. In contrast, ectopic expression of a constitutive activate form of Raf Δ reduced IL-1-induced NF- κ B activation, and this inhibition was completely reversed by PD98059. Interestingly, PD98059 sustained IL-1-induced NF- κ B DNA binding activity by an electrophoretic mobility shift assay and also I κ B α degradation, presumably by augmenting and sustaining the proteasome activation. Concomitantly, two NF- κ B dependent genes, A20 and I κ B α expression were prolonged with PD98059. These data suggested that MEK-ERK pathway exerts a regulatory effect on NF- κ B activation, providing a novel insight on the role of MEK-ERK pathway.

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Involvement of p38 in the regulation of inflammatory and Th1/Th2 cytokines

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p38, a stress activated MAPK (mitogen activated protein kinase), plays a crucial role in stress and inflammatory responses. In order to investigate the functional role of p38 MAPK signaling pathway with respect to cytokines,

a series of quantitative high content screening methods, the Multiplex Bead Immunoassay (MBI), were developed. The MBI technology involves the use of a Luminex 100™ analyzer, associated software and fluorescently encoded microspheres. Each microsphere is labeled with a distinguishable fluorophore that allows it to be assigned or gated to a particular region of the scanner. Antibodies specific for the protein of interest (here p38 or cytokines) are covalently linked to beads of a unique fluorescent region. The combination of different beads allows the user to simultaneously measure various protein markers of interest. Sensitivity and specificity for the multiplexed assays are comparable to the results from conventional immunoassays. Stimulation of Jurkat cells with PMA/Ca²⁺ resulted in activation of p38 which was detected by increase in phosphorylation of p38. Activation of p38MAPK correlates closely with the stimulation of T cell proliferation and production of Th1/Th2 cytokines such as IL-2, IL-4, IFN- γ and inflammatory cytokines such as HIL1 β , TNF α and GM-CSF. These data support the idea that p38 MAPK plays a central role in regulating the production of and responsiveness to pro-inflammatory and Th1/Th2 cytokines.

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LPS Signaled ERK Activity is Augmented by Substance P in Human PMN.

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The neurokinin, Substance P (SP), is a mediator of inflammation with direct effects on neutrophils (PMN). However, the signal pathways mediating these effects are unclear. Since SP signals activation of extracellular signal related kinase (ERK) we hypothesize that it may prime PMN for augmented ERK Mitogen Activated Protein Kinase (MAPK) activity during co-stimulation with other stimuli. To test this human PMN (isolated from healthy volunteers by gradient centrifugation and RBC sedimentation) were stimulated in vitro with bacterial lipopolysaccharide (LPS), a known activator of the ERK MAPK in PMN, with and without co-stimulation by SP. At various time points cultured PMN were lysed and MEK 1/2 phosphorylation (phospho-specific western blot) and ERK activity (cell free kinase assay) were assessed. SP augments LPS signaled ERK activity in human PMN. SP rapidly phosphorylates MEK 1/2 whereas LPS signaled MEK 1/2 activity is slower. Time course experiments indicate that MEK 1/2 phosphorylation is sustained in SP + LPS stimulated PMN compared to either signal alone. We conclude that SP signals MEK/ERK cascade activity in human PMN and augments LPS signaled ERK activity. Augmented ERK activity may result from sustained MEK 1/2 phosphorylation as was observed during co-stimulation in our experiments. Alternatively, effects on ERK regulatory phosphatases may also participate in SP modulation of this cascade. These results support the role of SP as a significant inflammatory mediator.

JAK/STAT (69-70)

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Definition of Tyk2-binding domains in Platelet-Activating Factor Receptor.

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Platelet-Activating Factor (PAF) activates the Jak/STAT pathway via the PAF receptor, a member of the seven transmembrane receptor family, in a G-protein-independent manner. Tyk2 is constitutively associated with the receptor and is essential for PAFR promoter activation. In order to define the binding domains for Tyk2, we first used PAFR C-terminal deletion mutants to show that M311Stop and T305Stop mutants precipitated Tyk2, suggesting that the C-terminus of the receptor was not necessary for Tyk2 binding. GST-fusion proteins, encoding 2nd and 3rd intracellular loops as well as the C-terminus of PAFR, precipitated both WT and a deletion mutant of Tyk2 suggesting the existence of multiple Tyk2-binding sites on the PAF receptor. Minigene constructs, encoding PAFR intracellular loops were transiently

transfected with PAFR and Tyk2 into COS-7 cells. In co-immunoprecipitation experiments, the minigene encoding the 1st intracellular loop of PAFR increased binding of Tyk2 to PAFR, while the second and the third loops had no effect. In luciferase assays, the 2nd intracellular loop as well as the C-terminus of the receptor, or the 1st and the 3rd loops together, inhibited PAFR promoter activation. PAFR mutants F40G, L43F, P45A, F49G bound Tyk2 similarly to WT PAFR, however L43F and M311Stop showed a 36% and 44% decrease in PAFR promoter activation, respectively. In summary, our results indicate that PAFR contains several binding sites for Tyk2, but it is the 2nd intracellular loop and the C-terminal tail of the receptor which are important for Tyk2 function.

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JAB/SOCS1/SSI-1 is an IL-2-induced inhibitor of IL-2 signaling

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JAB/SOCS1/SSI-1 is an SH2-domain-containing protein that is induced by and negatively regulates signaling by a number of cytokines including IL-4, IL-6, IFN- γ , prolactin, growth hormone, and erythropoietin. We analyzed the role of JAB/SOCS1/SSI-1 in IL-2 signaling. We show that JAB/SOCS1/SSI-1 is strongly induced by IL-2 in peripheral blood T cells and that JAB/SOCS1/SSI-1 overexpression strongly inhibits IL-2-induced Stat5 phosphorylation and transcriptional activity. In co-transfection experiments, JAB/SOCS1/SSI-1 associates with both Jak1 and Jak3; however, JAB/SOCS1/SSI-1 had a greater effect on Jak1 tyrosine phosphorylation and kinase activity. JAB/SOCS1/SSI-1 also interacts with IL-2R β and this interaction requires the A-region (residues 313-382) of IL-2R β ; however, this interaction was not essential for the inhibitory action of JAB. Thus, JAB/SOCS1/SSI-1 is an IL-2-induced inhibitor of IL-2-signaling which functions by inhibiting Jak kinase activity. This suggests an important role for JAB/SOCS1/SSI-1 in regulating T cell responses.

SMADs (71)

71

Expression of Inhibitory Smad7 in Experimental Uveitis

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Mechanisms of initiation and perpetuation of intraocular inflammation have not been fully elucidated. We have shown that the transcription factor nuclear factor-kappa B (NF- κ B) is activated during uveitis and regulates the transcription of several proinflammatory genes important in uveitis. Transforming growth factor beta (TGF β) is a multifunctional cytokine found in the normal eye; however, aqueous TGF β 2 levels decrease during active inflammation. Furthermore, TGF β 2 inhibits NF- κ B activation in endotoxin-induced uveitis (EIU). In the present study, we investigated the role of the inhibitory Smad7 in TGF β 2-induced suppression of inflammatory gene expression in vivo (EIU) and in vitro (IL-1 β -stimulated iris-ciliary epithelial monolayers). TGF β 2 and smad expression were examined by ELISA, western analysis, or immunohistochemistry. In addition to decreased aqueous TGF β 2 levels, protein expression in inflamed iris-ciliary body tissue exhibited decreased TGF β 2 during peak inflammation (24hr). Immunohistochemistry revealed that constitutive, basal Smad7 protein was only slightly expressed, but appeared in a time-dependent manner with expression most intense at peak inflammation in the ciliary epithelial cells. Furthermore, induction of Smad7 protein correlated with decreased TGF β 2 protein and progression of clinical uveitis. Characterization of the role of Smad7 and the interaction between opposing I κ B/NF- κ B and TGF β /SMAD signaling pathways will provide information critical to understanding the pathogenesis of intraocular inflammation and designing new and effective therapeutics.

NOVEL CYTOKINES AND CYTOKINE FUNCTION (72-77)

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DISTINCT SIGNAL TRANSDUCTION PATHWAYS INDUCED BY IL-2 AND IL-2/15R β 2 AGONISTS

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IL-2/15R β agonist p1-30 (containing amino acids 1 to 30, covering the entire α helix A of IL-2) spontaneously folds into an α -helical homotetramer mimicking the quaternary structure of an hemopoietin. P1-30 and other IL-2 derived analogs induce LAK cells and preferentially activate CD8 low lymphocytes and natural killer cells, which constitutively express IL-2R β . We demonstrate that these neocytokines interact with a previously undescribed dimeric form of IL-2/15R β . In agreement with its binding to IL-2/15R β , p1-30 activates Shc and p56lck but unlike IL-2, fails to activate Jak-1, Jak-3 and STAT5. Unexpectedly, we also show that p1-30 activates Tyk-2 thus suggesting that IL-2/15R β may bind to different Jak kinases depending on its oligomerization. At the molecular level, p1-30 alone induces the anti-apoptotic molecule bcl-2 but does not influence mRNA expression of c-myc, c-jun and c-fos oncogenes. In contrast, p1-30 enhances IL-2-driven expression of these oncogenes. At the receptor level, overexpression IL-2R α selectively inhibits p1-30 R formation and impedes the synergistic effects obtained with IL-2. We also demonstrate the ability of p1-30 to act in synergy with IL-4, IL-9 and IL-15. Altogether our data demonstrate that p1-30R (IL-2/15R β)₂ and intermediate affinity IL-2/15 R (IL-2/15R β) γ , induce distinct signal transduction pathways that may act in synergy.

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Expression of alternative spliced variants of cytokine mRNA in human erythroid cells.

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In the previous researches we found that mRNA expression and cytokines production are performed by human and mouse nucleated erythroid cells, in particular IL-4 and IL-6. The data of last 5-7 years has proved the fact of mRNA alternative splicing in formation IL-4 and IL-6 isoforms in immune system cells. The purpose of the present research was study of alternative variants cytokines mRNA expression in erythroid cells. We have isolated nucleated erythroid cells from human bone marrow of adult donors and fetal liver. To detect IL-4 and IL-6 mRNA expression, we have used a RT-PCR method. Selected PCR primers allowed amplification of cytokine sequences from first till fourth exons. Two RT-PCR product were detected for both IL-4 and IL-6. Alternative IL-4 mRNA variant which we detected is corresponded to IL-482 variant, described earlier by Alms V.J. The smaller IL-6 RT-PCR product is corresponded to IL-6-isoform expressed in PBMC and described by Kestler D.P. The role of the alternative forms of IL-4 and IL-6 produced by erythroid lineage in the regulation of haemato- and immunopoiesis in human bone marrow and fetal liver is discussed.

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IL-1H4 (IL-1F7), a novel member of the interleukin-1 gene family, is regulated by LPS and induces IL-8 but not IFN γ

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Recently six new members of the interleukin-1 (IL-1) gene family have been discovered from expressed sequence tag data base searches. These proteins share significant amino acid homology with the IL-1 receptor antagonist (IL-1Ra) and IL-1 β but the biological function is presently unknown. Based on

their structure they may act as agonistic or antagonistic ligands for members of the IL-1 receptor family. Three variants of the IL-1 homologue IL-1H4 with different N-termini have been described. IL-1H4 has been reported to bind the IL-18 receptor α -chain (IL-18Ra). However, unlike IL-18, we did not observe stimulation of IFN γ by IL-1H4 in the presence of IL-12 using NKO cells, human PBMC or human whole blood cultures. In contrast, IL-1H4 weakly stimulated IL-8 (2-fold) in human whole blood cultures which was independent on costimulatory signals such as IL-12 or LPS. No difference in activity was seen between IL-1H4 before or after the cleavage of an N-terminal propeptide. In order to study the expression of IL-1H4 at the protein level, we generated stable murine monocytic RAW 264.7 transfectants of the human IL-1H4 under the CMV-promotor. Transfected IL-1H4 is mainly produced intracellularly but also found in the cell culture supernatant with a molecular weight of 28 kDa. Both intracellular and extracellular production of IL-1H4 is markedly upregulated by LPS. Assuming constitutive activity of the CMV-promotor in the transfected RAW 264.7 cells, LPS posttranscriptionally increased IL-1H4 protein synthesis. In conclusion IL-1H4 does not induce IFN γ production, but is regulated similarly to known members of the IL-1 family.

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IL21 Receptor Expression and Modulation of Gene Transcription by Interleukin 21.

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Interleukin 21, made by activated CD4 T cells, exhibits effects alone and in combination with other cytokines on many lymphoid populations, including CD4 and CD8 T cells, B cells and NK cells. To understand this potentially broad role of IL21 in the immune system, we sought to characterize the effects of IL21 and other cytokines on IL21 receptor (IL21R) expression in different lymphoid subsets. Using both RNase protection and gene chip analysis on CD19+ purified B cells, we observed high constitutive expression of IL21R which could be further upregulated by IL21 or IL4 at 4 hours. We also studied the effects of IL21 on modulation of gene expression using DNA microarray chip technology. RNA was isolated from purified IL21-treated CD19+ B cells at different time points and hybridized to DNA arrays (gene chip). In B cells treated for 4 hours with IL21, we observed modulation of genes associated with activation, cell cycle progression, cytokine response and signaling. In 2D6 T cells, a murine cell line with constitutive expression of IL21R, 4h treatment with IL21 showed increased mRNA expression for several members of the Stat family as well as increased IL21R. RPA results showed that IL21R could also be upregulated in 2D6 cells in response to IL12, IL15 or IL21. These results suggest that IL21 has significant effects in modulation of genes associated with activation, signaling and cytokine responses.

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IL21 blocks IL15-induced NK cell expansion and enhances IFN γ production

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IL21, a cytokine produced by activated T cells, has homology to IL2 and IL15. It acts through a γ common chain-associated receptor that is widely expressed in lymphoid organs. IL21 enhances various lymphocyte responses in human systems, including NK cell maturation and activation, and T and B cell proliferation (Parrish-Novak et al., 2000). Experiments were done to further study the role of IL21 in murine NK cell and cytokine-driven T cell activation responses. IL21 was found to be a weak growth factor for mouse splenic NK cells, but a potent antagonist of IL15-induced NK cell expansion, reducing outgrowth by 80-95%. On a per cell basis, cytotoxicity was not impaired and IFN γ production was enhanced. IL21 also blocked the antigen non-specific, IL15-dependent induction of CD8+ memory-phenotype T cells, and expression of the cytokine receptors CD25, CD122, and CD119, while stimulating production of IFN γ by cytokine-activated T cells. The antagonism of IL15

effects is most likely due to competition between IL21 and IL15 for the shared γ common receptor chain. The IFN γ -inducing activity of IL21 is consistent with its enhancement of proliferation, cytokine production, and cytotoxicity by antigen-specific CD8+ T cells. During the course of an immune response, the development and mobilization of antigen-specific T cells coincides with the abatement of innate responses. By stimulating antigen-specific T cells while antagonizing NK cell expansion, IL21 may be a key facilitator of this transition.

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HYPER-6: A NEW DESIGNER CYTOKINE MODIFIED MELANOMA VACCINE - CLINICAL RESULTS OF A PHASE II STUDY.

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67 melanoma patients (AJCC III-IV) received genetically modified tumor vaccine (GMTV). GMTV consisted of irradiated Mich-1 melanoma cells transduced with Hyper-6 gene using retroviral double copy dicistronic vector. The toxicity of escalating doses of GMTV was assessed in advanced patients. The vaccine was locally and systemically well tolerated. In no instance adverse reactions necessitated a dose reduction of the vaccine or the withdrawal from the study. The vaccination frequency had no apparent effect on the reaction intensity. In the II phase objective clinical responses and overall survival were evaluated. Objective clinical responses were evaluable in 67 patients with measurable metastatic disease. Clinical responses (CR+PR+minor responses) were noticed in 18 (27%) patients. Regressions of melanoma metastases were seen in soft tissue and lungs. In comparison to historical control (case-control study) overall survival has been prolonged (log-rank test, $p < 0.05$). The 2-year survival was 30% in the vaccinated group and 6% in the control group. The survival of responders was better than non-responders. We are about to run a double-blind placebo, phase III randomized, prospective multicentre study to verify the clinical effectiveness of the Hyper-6 melanoma vaccine.

INTERFERONS AND IFN RECEPTORS (78-83)

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PEG Intron and interferon alfa-2b are comparable in regulating gene-expression for relevant anti-viral immuno-responsiveness.

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PEG Intron consists of a predominantly single unit of mono-methoxypolyethylene glycol covalently linked to interferon alfa-2b (IFN- α 2b). We wanted to profile the anti-viral comparability between PEG Intron and IFN- α 2b on the basis of early gene transcripts of IFN- α inducible genes. PBMC cultures from 5 normal healthy donors were treated with PEG Intron at 20 or 200 U/ml, IFN- α 2b at 20 or 200 U/ml, or IFN γ at 0.25 ng/ml for 4 hours. The transcripts for 15 different interferon alpha inducible genes were quantitated using two-step real-time PCR (ABI 7700). Not surprising, the direct anti-viral-activity mediated transcripts were highly induced, 2'-5' OAS (11.1 \pm 30.7 fold) and PKR (4.6 \pm 11.2 fold). However, the highest transcript fold-inductions were observed for the IFN γ cytokine system, i.e., IFN γ inducing cytokine ISG-15 (20.5 \pm 64.3 fold) and IFN γ (23.5 \pm 49.4 fold). The transcript for the cytolytic-activity protein perforin was also highly up-regulated (6.6 \pm 9.9 fold). We observed a dose-dependent response of gene expression to interferon treatment and were able to discriminate between the type I and type II interferon-regulation of gene transcription. At both 20 and 200 U/ml, there was no significant difference between IFN- α 2b and PEG Intron in the induction of mRNA transcripts for IFN γ , ISG-15, PKR, 2'-5' OAS, STAT1, STAT2, IRF-1, IRF-2, and tryptophanyl-tRNA synthetase. When PEG Intron and IFN- α 2b were dosed at levels of equivalent activity in the CPE anti-viral assay, equipotency was observed with no significant agonist or antagonist activity present. These data demonstrate that PEG Intron and IFN- α 2b are comparable for the induction of relevant early gene transcripts for both direct anti-viral activity and for immune-mediated anti-viral activity.

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Interferon Signaling Requires Specific Phosphotyrosines Located Within the Intracellular Domain of IFNAR2c

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In this report, we identify two specific phosphotyrosine residues contained within the cytoplasmic domain of the type I interferon receptor chain 2 (IFNAR2c) that are obligatory for interferon (IFN) dependent signaling. A human cell line lacking IFNAR2c (U5A) was used to determine the role of phosphotyrosine residues in regulating IFN dependent signaling. Stable U5A cell transfectants expressing various IFNAR2c tyrosine mutations were tested for their ability to induce STAT1 and STAT2 activation, ISGF3 transcriptional complex formation, gene expression and the regulation of cell growth in response to IFN. Elimination of single tyrosines at Tyr269, Tyr316, Tyr318, Tyr337 or Tyr512 had no effect on IFN signaling suggesting that no single tyrosine is critical for IFN dependent receptor activation. Furthermore, IFNAR2c having either the five proximal (Tyr269, 306, 316, 318, 337) or two distal (Tyr 411, 512) tyrosine residues substituted with phenylalanine continued to be responsive to IFN stimulation. The complete substitution of all seven cytoplasmic tyrosine residues of IFNAR2c resulted in a nonfunctional receptor. Most importantly, the addition of a single tyrosine at either Tyr337 or Tyr512 was sufficient to induce a full response to IFN whereas other IFNAR2c mutants having only a single tyrosine at Tyr269, Tyr306, Tyr316, Tyr318 or Tyr411 were unable to respond. Surprisingly, we have found that IFN dependent signaling is mediated through the redundant usage of a single phosphotyrosine at either Tyr337 or Tyr512.

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A Comparison of the Anti-Viral Efficacy of Murine Type I IFN Transgenes Against Herpes Simplex Virus Type 1

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The present study compared the efficacy of type I IFN transgenes including IFN- α 1, - α 4, - α 5, - α 6, - α 9, and - β against HSV-1 infection *in vitro*. Cells transfected with the type I IFN transgenes produced similar amounts of IFN as measured by ELISA (range of 728-1137 pg/ml) and bioassay (1210-1550 units/ml). Cells transfected with the murine IFN- β transgene exhibited the greatest reduction (30 fold) in viral titers whereas cells transfected with the murine IFN- α 5 showed a modest inhibitory effect (3 fold). Likewise, IFN- β was found to have the lowest IC₅₀ value (22 units/ml) whereas IFN- α 5 showed the highest IC₅₀ value (147 units/ml) against HSV-1. Real time PCR analysis of selective viral genes showed that cells transfected with the IFN- β transgene antagonized viral transcript levels to a greater extent compared to the IFN- α transgenes at 6 and 10 h post infection. Transfected cells showed reduced viral protein expression as well. Although cells transfected with the type I IFN transgenes showed comparable levels of OAS and PKR mRNA expression post infection and these levels were elevated above control plasmid transfected cells, the PKR inhibitor p58 mRNA level was repressed over 10 h in cells transfected with the IFN- β transgene while levels from the IFN- α transgene groups were only suppressed for 6 h. Collectively, these results show IFN- β is superior to IFN- α subtypes in antagonizing HSV-1 replication.

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WITHDRAWN

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Characterization of Pathways Promoting IFN- α/β Expression during In Vivo Viral Infections

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In vitro studies have revealed that IFN- α/β can induce itself in an autocrine amplification loop. Studies were initiated to evaluate the requirements of a functional IFN- α/β receptor for induction of IFN- α/β expression during *in vivo* viral infections, using bioassay and RT-PCR analyses. Infections of mice with both lymphocytic choriomeningitis virus (LCMV) or murine cytomegalovirus (MCMV) induced profound early IFN- α/β responses. Peak responses in wildtype mice were observed at 24-48 h after infections with LCMV and at 36 h after MCMV. The kinetics were extended during LCMV as compared to MCMV. Expression of IFN- α/β during infections of IFN- α/β R^{-/-} mice was significantly reduced during both infections. However, IFN- α/β R^{-/-} mice showed normal expression of IFN- α/β in the serum at 48 after infection with LCMV but never had a normal IFN- α/β response during MCMV infection. Expression of IFN- α/β subtype mRNA was shown to reflect the results obtained with the bioassay. These data demonstrate that the presence of virus alone is not enough to induce type I interferon expression *in vivo*, as shown by two different viral infections. Instead signaling through the IFN- α/β receptor is necessary for optimal induction. However, during infections with LCMV, alternative pathways take over in the absence of a functional IFN- α/β receptor. This indicates that, although induction of IFN- α/β with both viruses depends on the IFN- α/β receptor, different regulatory pathways modulate the IFN- α/β response under particular conditions of challenge.

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The 2',5'-oligoadenylate synthetase gene in mice

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The 2-5A system is known to be involved in the antiviral mechanisms of interferon (IFN). The 2',5'-oligoadenylate synthetase (2-5OAS) is one of IFN-induced proteins and forms a well conserved family. In humans, there are four classes of 2-5OAS genes, short, middle, and long form and one related protein (OAS-RP). The short form 2-5OAS has a set of the essential motifs for the 2-5OAS enzyme activity, OAS2 has two and OAS3 has three catalytic units. OAS-RP has a single OAS unit and two consecutive ubiquitin-like sequences in the carboxyl-terminal, but lacks 2-5OAS activity. In mice, however, 2-5OAS genes corresponding to the middle and long forms have not been identified. In this study, we have identified six novel 2-5OAS family genes in mice, which we named *OAS L6-L11*, by screening mouse genomic library and Expressed Sequence Tag (EST) database. *OAS L6-L8* are the members of the short form 2-5OAS gene, while *L11* is the middle form, *L10* is the long form, and *L9* corresponds to the *OAS-RP*. These genes display 52-65% amino acid identity to the corresponding human homologues. Nine 2-5OAS genes, except for two *OAS-RP* genes, exist within 220kb genomic region and form a cluster. These 2-5OAS genes express differently among different tissues, while all of these genes were induced by an IFN inducer, polyinosinic-polycytidylic acid (poly I:C). These results show that the mouse 2-5OAS gene family consists of at least eleven genes divided into four classes; seven short form, one middle form, one long form, and two *OAS-RP* genes. In mice, the short form and *OAS-RP* type genes, which occur as single genes in humans, are duplicated and multiplied.

NEUTROPHIL ACTIVATION (84-92)

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Oxidative Burst by Toll Like Receptors (TLR) and CD14 in Avian Heterophils Stimulated with Bacterial Components

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Cells belonging to the innate arm of the immune system must recognize antigens by pattern recognition receptors (PRR) that recognize pathogen-associated molecular patterns (PAMP) such as lipopolysaccharide (LPS) found in the cell wall of gram-negative bacteria and lipoteichoic acid (LTA) found in the cell wall of gram-positive bacteria. Experiments were conducted to stimulate oxidative burst of chicken heterophils with gram-negative *Salmonella enteritidis* (SE) or gram-positive *Staphylococcus aureus* (SA). Heterophils isolated from neonatal chicks were exposed to SE or SA for 30 minutes and luminol-dependent chemiluminescence was used to quantitate oxidative burst. A significant increase in oxidative burst was observed from heterophils when stimulated with both SE (48%) and SA (114%). We then evaluated the effect of purified LTA and LPS on oxidative burst of avian heterophils and also observed a significant increase in oxidative burst from heterophils when stimulated with LPS (25%) and LTA (89%). Further experiments using polymyxin B (47%), anti-human CD14 Ab (61%), anti-human TLR2 Ab (71%), and anti-human TLR4 Ab (72%) as antagonists to LPS were shown to significantly reduce oxidative burst. Therefore, these data demonstrate the interaction of PAMPs from gram positive and gram-negative bacteria with avian heterophils stimulate oxidative burst via TLR2, TLR4, and CD14.

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Activation of Chicken Immune Cells by Unmethylated CpG Dinucleotide Motif of Bacterial DNA

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Unmethylated CpG dinucleotide motif is a Pathogen Associated Molecular Pattern (PAMP) of bacterial DNA. Recent studies have shown that vertebrate immune systems are able to recognize unmethylated CpG motifs and trigger protective immune responses. However, effects of CpG motif on avian immune responses have not previously been reported. Using synthetic oligonucleotides containing the CpG motif, we measured the immune responses of neonatal chicken peripheral blood heterophils and mononuclear cells and of the chicken macrophage cell line (HD-11) to CpG dinucleotide stimulation. RT-PCR demonstrates that the CpG dinucleotide motif is a strong stimulator of inflammatory cytokine IL-1 expression in heterophils and IFN- γ expression in mononuclear cells. Incubation of heterophils and HD-11 cells with CpG dinucleotide significantly increases the level of oxidative burst and nitric oxide production, respectively. Our results demonstrate that the CpG motif is able to stimulate proinflammatory responses, up-regulate cytokine expression and increase nitric oxide production and oxidative burst in chicken immune cells.

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PRESERVATION OF THE PATTERN OF TYROSINE PHOSPHORYLATION IN HUMAN NEUTROPHIL LYSATES. II. A sequential lysis protocol for the analysis of tyrosine phosphorylation-dependent signaling

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In stimulated neutrophils, the majority of tyrosine phosphorylated proteins are concentrated in Triton X-100 or NP-40- insoluble fractions. Most immunobiochemical studies, however, whose objective is to study the functional relevance of tyrosine phosphorylation are performed using the supernatants of cells lysed in non-ionic detergent-containing buffers (RIPA lysis buffers). This observation prompted us to develop an alternative lysis protocol. We established a procedure involving the sequential lysis of neutrophils in buffers of increasing tonicity that not only preserved and solubilized tyrosine phosphorylated proteins but also retained their enzymatic activity. The sequential lysis of neutrophils in hypotonic, isotonic and hypertonic buffers containing non-ionic detergents resulted in the solubilization of a significant fraction of tyrosine phosphorylated proteins. Furthermore, we observed that in stimulated neutrophils, Lyn activity was enhanced in the soluble fraction recovered from the hypertonic fraction, but not from that of the first hypotonic lysis. The distribution of tyrosine phosphorylated proteins between the NP-40 soluble and insoluble fractions was both substrate- and agonist-dependent. In neutrophils stimulated with MSU crystals or by CD32 ligation, the tyrosine phosphorylated proteins were mostly insoluble. On the other hand, in fMLP or GM-CSF- treated cells, they were more equally distributed between the two fractions. The results of this study provide a new experimental procedure for the investigation of tyrosine phosphorylation pathways in activated human neutrophils which may also be applicable to other cell types.

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Diverging effects of γ interferon and TNF α on polymorphonuclear neutrophils (PMN): induction of cytokines synthesis, receptor expression and differentiation to antigen-presenting cells.

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The constitutive apoptosis of PMN is delayed by a number of stimuli, including γ IFN or TNF α in low doses. We now describe that escape from apoptosis is associated with phenotypical and functional alterations: within 24 h, PMN appeared more elongated and extended pseudopodia; synthesis of interleukin 8 (IL-8), macrophage inflammatory proteins (MIP-1) α and MIP-1 β , TNF α and TNF β and ENA78 was induced as measured by quantitative RT-PCR; the surface-receptors CD11b/CD18, CD14, CD64 and CD66b were

up-regulated. Of special interest was the generation of CD83, a receptor thought to be specific for dendritic cells. CD83 was predominantly up-regulated by TNF α , which was the more potent activator also for the other parameters. Escape from apoptosis and the stimulation of receptor expression could be inhibited by interfering with NF- κ B activation. While these data suggest similar signaling pathways, there was an important difference: only γ IFN, but not TNF α , induced synthesis of MHC class II antigens and of the co-stimulatory receptors CD80, and CD86. Moreover, only after culture with γ IFN, PMN acquired the ability to present peptide antigens in a MHC class II restricted manner. In accordance with our in vitro data we found upregulation of CD11b/CD18, CD14, and CD66b on PMN of patients with acute bacterial infections or chronic inflammatory disease, such as Wegener's granulomatosis. However, only the latter expressed MHC class II antigens, CD80, and CD86. PMN of patients with acute bacterial infection, expressed CD83, which was never found during chronic inflammation. These data are compatible with the in vitro finding that TNF α and γ IFN have overlapping, but distinct effects on PMN and that receptor expression on PMN might reflect cytokine patterns associated with the respective disease.

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Expression of granzyme B and perforin in polymorphonuclear neutrophils (PMN): identification and functional analysis

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Polymorphonuclear neutrophils are in the first line of defence against bacterial infection. In addition to oxygen or nitrogen radicals which are readily generated upon stimulation, they also contain an abundance of preformed cytotoxic or bactericidal proteins, which are released by degranulation. We now found that PMN contain preformed perforin and granzyme B, molecules known as cytotoxic entities of NK-cells and cytotoxic T-cells. By confocal laser scan microscopy intracellular deposits of both, perforin and granzyme, B were detected, though not in the same distribution. By immunoprecipitation of cellular lysates perforin and granzyme B were detected in freshly isolated PMN and to a lesser degree in PMN cultivated for 24 h with or without γ interferon. By RT-PCR message for both perforin and granzyme B could be detected; de novo protein synthesis, however, was not seen. Lysates of PMN prepared by repeated freezing and thawing cycles contained lytic activity for chicken erythrocytes and enzymatic activity, as tested by a granzyme B-specific substrate conversion. Moreover, lysates of PMN induced apoptosis of Jurkat cells which were used as a target. Within 60 minutes the first signs for apoptosis appeared, as judged by Annexin V binding and propidium iodide staining. After 3 to 4 hours the majority of cells was dead determined by their failure to exclude trypan blue. Killing of Jurkat cells was inhibited by Z-Val-Ala-Asp-OMe-(fluoromethylketone) considered to be a specific inhibitor for granzyme B. Taken together our data indicate that PMN express perforin and granzyme B. Though the role of these molecules in PMN has not yet been identified, it is reasonable to assume that they participate in antibody-dependent-cellular cytotoxicity (ADCC), a long-known function of PMN.

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Stealth LPS from Prevotella species

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In our dental clinic, we saw patients with early onset periodontal disease or necrotic endodontic abscesses in which the predominate microbe was a member of the genus Prevotella. In vitro, these gram-negative oral pathogens failed to prime neutrophils or monocytes for release of oxygen radicals or cytokines, prompting us to refer to Prevotella as a "stealth" pathogen. We studied the LPS from Prevotella denticola (Pd) and Prevotella intermedia (Pi), compared with LPS from Actinobacillus actinomycetemcomitans (Aa), Fusobacterium nucleatum (Fn), and Escherichia coli (Ec). LPS was extracted with hot phenol-water, and tested by Limulus assay. Pd LPS showed Limulus activity comparable to Aa LPS. However, when Pd LPS was tested for ability to prime neutrophils or monocytes for enhanced release of superoxide, it had < 1% of the expected activity. For example, untreated neutrophils produced 7.3

± 0.1 nmol superoxide per million neutrophils when triggered with FMLP, neutrophils primed with 1 ng/ml of Aa LPS produced 49.8 ± 7.7 nmol, and neutrophils primed with 1 ng/ml of Pd LPS produced 8.0 ± 0.1 nmol (means \pm SE). Pd LPS (0.1-100 ng/ml) also failed to induce monocyte secretion of IL-1 β or TNF α , whereas Aa LPS was active at 0.3 ng/ml. We prepared lipid A from LPS by hydrolysis in 1.5% acetic acid at 100°C for 2 h. Lipid A from Aa, Fn, and Ec showed the expected phagocyte priming ability (half-maximal priming < 1 ng/ml), but lipid A preparations from Pd or Pi had almost no ability to prime phagocytes (half-maximal priming > 300 ng/ml). We analyzed the lipid A by MALDI mass spectrometry. Lipid A from Ec and Fn showed the expected molecular ion at 1796.4 Da. Aa, which has a C14 fatty acid vs C12 in Ec, gave the expected ion at 1824.2 Da. Ions reflecting loss of fatty acids were observed, as expected. However, with Pi and Pd, no ions indicative of a normal lipid A were observed. We conclude that Prevotella species lack normal lipid A and normal LPS, and thus may avoid being recognized and killed by phagocytes. Supported by NIH grant DE05494.

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GM-CSF AND TNF α ENHANCE ACTIVATION OF NEUTROPHIL PHOSPHOLIPASE D (PLD) TRIGGERED BY PHAGOCYTOSIS

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Activation of PLD by chemoattractants, chemokines, and phagocytic particles in neutrophils is linked to the regulation of effector functions, e.g. the respiratory burst. Studies have shown that pro-inflammatory cytokines enhance PLD activation and the respiratory burst triggered by chemokines and chemoattractants. We examined whether this priming effect extends to activation of PLD by phagocytosis, using opsonized zymosan (OPZ) as the phagocytic stimulus. Both granulocyte/macrophage colony stimulating factor (GM-CSF; 400 pM, 25 min) and tumor necrosis factor α (TNF α ; 200 U/ml, 30 min) enhanced OPZ-triggered PLD activity (measured as phosphatidylethanol production) by ~2-fold. In contrast, G-CSF (10 or 25 ng/ml, 20 min) or interferon γ (100 U/ml, 60 min) had no effect. After cytokine treatment, levels of OPZ-stimulated phosphatidic acid (PA) increased more rapidly than in buffer-treated cells. Cytokine treatment had minimal effects on the levels of diglyceride, suggesting that the main effect of priming was to increase the amount of PA. In macrophages, PLD activation is known to regulate phagocytosis of OPZ, suggesting that cytokine priming enhances uptake of phagocytic particles. We are now studying if enhancement of PLD activation by GM-CSF and TNF α leads to enhanced activation of the respiratory burst by OPZ. Thus, PLD activation may be a key signalling target for GM-CSF and TNF α , resulting in enhanced ability of neutrophils to phagocytize and destroy microorganisms. Supported by grants from the March of Dimes Res. Fdn. (FY00-698) and NIH (R01AI22564).

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INHIBITORY ACTIONS OF GLUCOSAMINE, A THERAPEUTIC AGENT FOR OSTEOARTHRITIS, ON THE FUNCTIONS OF NEUTROPHILS

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Glucosamine, an amino monosaccharide naturally occurring in the connective and cartilage tissues, contributes to maintaining the strength, flexibility and elasticity of these tissues. In recent years, glucosamine has been widely used to treat osteoarthritis in humans and animal models. Neutrophils, which usually function as the primary defenders in bacterial infections, are also implicated in the destructive inflammatory responses in arthritis. In this study, we evaluated the effects of glucosamine on neutrophil functions using human peripheral blood neutrophils. Glucosamine (0.01-1 mM) dose-dependently suppressed the superoxide generation induced by fMLP (formyl-Met-Leu-Phe) or C3bi-opsonized zymosan, and inhibited the phagocytosis of C3bi-opsonized zymosan or IgG-opsonized latex particles. Furthermore, glucosamine inhibited the release of granule enzyme lysozyme from phagocytosing neutrophils, and

suppressed neutrophils chemotaxis towards C5a. In addition, glucosamine significantly inhibited the fMLP-induced upregulation of CD11b, polymerization of actin, and phosphorylation of p38 MAPK. In contrast, N-acetylglucosamine, an analogue of glucosamine, did not affect the superoxide generation, phagocytosis, granule enzyme release, chemotaxis, CD11b expression, actin polymerization, and p38 MAPK phosphorylation. Together, these observations likely suggest that glucosamine possibly suppresses the neutrophil functions, thereby exhibiting anti-inflammatory actions in arthritis.

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Transferrin-derived phagocytosis activators (MAPPs): characterization of the transferrin microheterogeneity

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Macromolecular activators of phagocytosis from platelets (MAPPs: I-MAPP, 300kDa and s-MAPP, 150kDa) consist of polymeric (dimer and tetramer) transferrin and a 800 Da substance and enhance neutrophilic phagocytosis via the Fc γ receptors when neutrophils are attached on the surface separately. They can be produced in vitro using thrombin-treated platelet lysate and transferrin (TF) polymerized by glutaraldehyde (Biochem Biophys Res Com, 270,377,2000). It is known that microheterogeneities exist in TF mainly according to the differences in the carbohydrate residues. The purpose of this study is to characterize and to purify partially the TF which is used to form MAPP (MAPP-TF). Commercially available human TF (Sigma) were fractionated by two steps of preparative isoelectric focusing using Roto-Lyte and Bio-Lyte as ampholytes. The pI of MAPP-TF was determined to be 5.0 and it was suggested that homo-dimer and homo-tetramer of these transferrin could form phagocytosis activators corresponding to s-MAPP and I-MAPP respectively. The yield of MAPP-TF was 0.72% of the starting material and the effective maximum dilution of the MAPP produced increased from 10^2 (starting material) to 10^9 . The plasma obtained from healthy volunteers also showed existence of MAPP-TF at the fraction with the pI, 5.0. These results suggest that MAPP-TF is a highly sialylated TF and a minority in transferrin family.

MACROPHAGE ACTIVATION (93-105)

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Regulation of Cyclooxygenase (Cox)-2 Expression in macrophages: Signaling pathways of the RON Receptor Tyrosine Kinase that inhibit LPS-induced Cox-2 Gene Transcription

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Inflammatory mediators produced by macrophages such as Cox-2 and iNOS play an important role in eliciting inflammatory responses. Inhibition of Cox-2 and iNOS expression has been proved to be critical in reducing inflammatory reactions during bacterial infection and tissue injury. We report here that macrophage stimulating protein (MSP) inhibits not only iNOS but also Cox-2 expression in murine Raw264.7 macrophages treated with LPS and inflammatory cytokines such as IFN- γ . The inhibitory effect of MSP is mediated by the RON receptor tyrosine kinase that affects the early stages of Cox-2 synthesis and its mRNA expression. By analyzing signaling proteins that are involved in Cox-2 and iNOS expression, we found that RON activation significantly reduces the amounts of MyD88 adaptor protein associated with Toll-like receptor 4 in LPS-stimulated Raw264.7 cells. Moreover, the stabilities of I κ B- α and I κ B- β that controls NF- κ B activation were also dramatically increased. Western blot analysis confirmed that phosphorylation of I κ B- α and I κ B- β was significantly reduced in LPS-treated Raw264.7 cells that expressing a constitutively active RON variant, indicating that LPS-

induced IKK activities might be affected after RON activation. In conclusion, our results suggest that MSP is a potent inhibitor that blocks macrophage iNOS and Cox-2 expression. The mechanisms by which MSP-induced iNOS or Cox-2 inhibition might be mediated by a unique signaling pathway(s) of RON that blocks the interaction of TLR-4 with adaptor protein MyD88 leading to the inhibition of NF- κ B activation. Supported by NIH R01 Grant AI-43516 to MHW

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Expression of cytokine-related genes in leukocytes infected with herpes simplex virus 1 and 2: comparison between resistant and susceptible mice strains

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Cytokines and chemokines play an important role in the first line of defence against infections. Moreover, these groups of proteins also contribute significantly to regulation of the acquired immune response. Therefore, knowledge about expression of cytokines, chemokines and other factors involved in their mechanisms of action may provide information about the immune response responsible for elimination of infectious agents and immune-mediated pathogenesis. Using cDNA arrays we have evaluated the pattern of expression of cytokines and cytokine-related genes by murine peritoneal cells infected with herpes simplex virus (HSV)-1 and 2. Moreover, we have compared the expression profiles of the resistant mice strain C57BL/6 and the susceptible strain BALB/c. The results are presented and discussed with emphasis on differences between the two HSV types and the two mice strains. The identified differences may provide information about host factors involved in clearance of virus infections and may also shed light on the subtle pathogenic differences between HSV-1 and HSV-2.

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PMA Inhibits Plasmin-mediated Release of Aggregated LDL from Macrophages.

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Aggregation of low density lipoproteins (LDL) may contribute to their retention in atherosclerotic lesions. Previously, we showed that aggregated LDL (AgLDL) induce and enter surface-connected compartments (SCC) in human monocyte-derived macrophages by a process we have named patocytosis. AgLDL were disaggregated and released from SCC of macrophages when exposed to human lipoprotein-deficient serum (LPDS). The serum factor that mediated AgLDL release and disaggregation was plasmin generated from plasminogen by macrophage uPA. Plasminogen could substitute for LPDS and produce similar macrophage release and disaggregation of AgLDL. We now show that activation of macrophages with PMA inhibits plasmin-mediated release of AgLDL from macrophages. Macrophages that had accumulated AgLDL for 5 h, and then were incubated 1 d with 10% LPDS or plasminogen (1 U/ml) \pm PMA (0.1 μ g/ml), released 57-60% less cholesterol and 63% less TCA-insoluble 125 I-AgLDL in the presence of PMA. PMA also inhibited by 57% trypsin-induced release of AgLDL cholesterol from macrophages. Electron microscopy showed that PMA did not cause SCC to close which could have trapped AgLDL within SCC and limited protease access to AgLDL. Rather, PMA stimulated lysosomal degradation of AgLDL by 2.5-fold. PMA-stimulated degradation of AgLDL was associated with a 2.5-fold increase in cholesterol esterification consistent with hydrolysis and re-esterification of AgLDL-derived cholesteryl ester. In conclusion, macrophage activation with PMA causes more of AgLDL that enters macrophage SCC to be metabolized by lysosomes, resulting in more cholesterol to be stored in macrophages, and less AgLDL to be available for plasmin-mediated release from macrophage SCC.

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SEROTONIN MODULATES THE PRODUCTION OF MEDIATORS BY ALVEOLAR MACROPHAGES.

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Serotonin, well known for its role in depression, has been shown to modulate some immune responses. Interestingly, plasma level of serotonin is increased in symptomatic asthmatic patients and the use of anti-depressant, known to reduce free level of serotonin, provoked a decrease in asthma symptoms and an increase in pulmonary function. Thus, we tested the hypothesis that serotonin modulate alveolar macrophage cytokine production contributing to the Th1/Th2 imbalance observed in asthma. Rat alveolar macrophage cell line (NR8383) was treated with different concentrations of serotonin (10^{-9} to 10^{-11} M) for 2 h prior their stimulation with LPS or BCG. Levels of macrophage inflammatory protein 1 α (MIP-1 α), TNF, IL-1 β , IL-10, IL-12, and nitric oxide (NO) were measured in cell free supernatants. Serotonin (10^{-9} and 10^{-10} M) significantly inhibited the production of MIP-1 α , TNF, and IL-12 whereas IL-1 β , IL-10, prostaglandin E_2 (PGE $_2$), and NO production was significantly increased. Inhibitors of cyclo-oxygenase and antibody to PGE $_2$ abrogated the inhibitory effect of serotonin on TNF production. These results suggest that serotonin may play a role in the modulation of the cytokine network contributing to asthma pathogenesis. This research was funded by Quebec Lung Association and FRSQ. undefined undefined undefined

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Multiple Distinct Signal Transduction Pathways are Induced by Toll-like Receptors 2 and 4

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Mammalian Toll-like receptor (TLR) proteins are pattern recognition receptors for a diverse array of bacterial and viral products. Gram-negative bacterial lipopolysaccharide (LPS) activates cells via Toll-like Receptor (TLR) 4, whereas bacterial lipoproteins and mycobacterial cell wall glycolipids (lipoarabinomannan (LAM) and mannosylated phosphatidylinositol (PIM)) activate cells via TLR2. Short-term culture filtrates of *M. tuberculosis* bacilli secrete a TLR2 agonist activity, termed Soluble Tuberculosis Factor (STF), that appear to contain PIM. We recently showed that stimulation of RAW 264.7 murine macrophages by LPS, LAM, STF, and PIM rapidly activated NF- κ B, AP-1 and MAP kinases. Here we show that signaling via TLR2 and TLR4 activates the Src-like protein kinases Lyn, a molecule known to be associated with lipid rafts. While Lyn and the LPS co-receptor CD14 are predominantly found in lipid rafts, TLR4 and other downstream mediators of TLR signaling (e.g. MyD88, IRAK, Tollip) were not found in these lipid rafts. Interestingly, activation of Lyn via TLR2 and TLR4 did not require MyD88. TLR2 and TLR4 engagement also activates the protein kinase Akt, and this activation could be blocked by specific inhibitors of phosphatidylinositol-3'-kinase (PI-3-K). Thus, activation of PI-3-K appears to be a response that is shared by engagement of TLR2 and TLR4. LPS, the mycobacterial glycolipids, and the OspC lipoprotein (a TLR2 agonist) all induced macrophages to secrete tumor necrosis factor α (TNF- α), whereas only LPS was capable of inducing nitric oxide (NO) and β interferon secretion. Together, these data show that different TLR proteins mediate the activation of distinct cellular responses, in spite of their shared capacities to activate NF- κ B, AP-1, MAP kinases, and PI-3-K.

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Roles of Toll-like Receptors in Immunity Against Mycobacteria

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Mammalian Toll-like receptor (TLR) proteins are pattern recognition receptors that mediate cellular activation by a wide variety of bacterial products. TLR activation leads to the expression of pro-inflammatory cytokines, chemokines, co-stimulatory molecules, and nitric oxide (NO) by macrophages. Thus, TLR-dependent cellular responses are likely to be required for successful immunity against bacterial pathogens. We previously showed that live *M. tuberculosis* (Mtb) bacteria can activate macrophages via both TLR2 and TLR4. We have now found that the induction of TNF- α secretion by Mtb-stimulated macrophages is mediated by these TLR proteins, although predominantly by TLR4. In contrast, NO production does not appear to be dependent on TLR signaling. This conclusion is based on the observation that Mtb-stimulated macrophages from MyD88-/- mice (i.e. mice that cannot signal via TLR proteins) did not secrete TNF- α , but did release normal levels of NO. Alternatively, Mtb-induced NO production may arise from a novel TLR-dependent and MyD88-independent signaling pathway. We subsequently sought to determine whether TLR2 and TLR4 participate in the control of mycobacterial infection in vivo, and whether these TLR proteins differentially mediate host responses. Using TLR4-deficient C3H/HeJ mice, we found that infection with live mycobacteria resulted in enhanced bacterial growth in vivo compared with normal C3H/OuJ mice. Furthermore, serum levels of IL-12p70 and IFN- γ were markedly lower in the infected C3H/HeJ mice compared with infected controls. Together, these studies indicate that TLR proteins mediate selected host responses against mycobacterial infection.

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Proteome of Monocytes Primed by LPS

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In our model of macrophage activation, monocytes exposed to LPS or to cytokines like IFN γ , IL-1 β , or TNF α in culture maintain an ability to produce high amounts of oxygen radicals in response to triggering with phorbol ester (primed monocytes). In contrast, monocytes not treated with LPS or cytokines lose their ability to generate oxygen radicals (quiet monocytes). In earlier work, we found that the serine protease inhibitor AEBSF could block the effect of LPS or cytokines. To further examine these monocyte responses, we performed a proteomic analysis. Monocytes were cultured with or without LPS and AEBSF, their phorbol ester-triggered release of superoxide was measured, viability and protein content were checked, and, if all were well, the monocytes were lysed in a solution containing urea, thiourea, CHAPS detergent, and dithiothreitol. The cellular proteins were separated by 2D gel electrophoresis. We compared the pattern of protein spots from primed versus quiet monocytes. Although most protein spots were identical in position and intensity, several spots appeared or disappeared in response to LPS. These changes in response to LPS were blocked by AEBSF. Protein spots of interest were cut from the gel, digested with trypsin, and the tryptic peptides eluted from a ZipTip C18 micro-column. The peptides were mixed with a matrix of α -cyano-4-hydroxycinnamic acid, and spotted onto a metal plate for analysis by MALDI-TOF mass spectrometry. We compared the exact molecular weights of the tryptic peptides with the predicted peptides from all known proteins, and were able to identify most of the interesting protein spots. Among the interesting proteins identified were the calgranulins. Calgranulin A, calgranulin B, and a phosphorylated form of calgranulin B were elevated in response to LPS, but this elevation was blocked by AEBSF. We conclude that, in monocytes, the calgranulins are closely associated with priming by LPS. Supported by NIH grant DE05494.

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Demonstration of a macrophage proliferation inhibition factor from human decidua distinct from transforming growth factor beta.

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The tissues comprising the deciduum contain a wide range of cytokines with diverse capabilities to influence the growth and activation of a range of cells. Thus, the placenta may prove to be a source of cytokines whose activity may

have important implications in disease states such as cancer where the normal regulation of cellular proliferation and differentiation has been lost. We have used decidua from human first trimester pregnancies to examine the biological activity of cytokines that affect the proliferation of mononuclear cells and particularly human monocytic leukaemia cells. Decidua were macerated, sonicated, ultracentrifuged and subjected to repeated ultrafiltration using nominal exclusion rates of 10,30, 100 and 300 Kda. After preliminary experiments, the < 10K and < 30KDa fractions were concentrated and subjected to high performance liquid chromatography. Fractions were then analysed for their ability to affect a variety of in vitro functional assays. Fractions were examined by SDS-PAGE and Western blotting. Results: Fractions 38-44 from the HPLC showed the maximum inhibition of mitogen stimulation. These fractions were pooled and their effect on a mixed lymphocyte reaction (MLR) studied. Again, the MLR was inhibited by the addition of 10%v/v decidual fraction. Since several studies have demonstrated the presence of TGF- β in human deciduas, we compared the effect of the DF with that of purified, recombinant human TGF- β . At 2ng/ml, TGF- β was shown to inhibit the proliferation of PHA stimulated PBMC and an one-way MLR. To investigate this further, we examined the effect of DF on the proliferation of the human cell lines Jurkat E6.1 and Mono Mac 6 and compared this to the activity of TGF- β . Again, both DF and TGF- β showed similar activities, inhibiting the replication of MM6 cells but not that of the human T cell leukaemia cell line, Jurkat E6.1. However, when the effect of these agents on the cell cycle distribution of the cell lines was examined, a distinct difference was observed. Whilst TGF- β was found to increase the number of cells in G0/G1 and in the pre-G0 region, the DF failed to show any effect on cell cycle distribution. Cultures of MM6 and E6.1 cells were then incubated with DF or TGF- β in the presence or absence of anti-TGF- β . The antibody neutralised the inhibition caused by TGF- β but did not alter the effect of DF. When SDS-page analysis was performed on a 12% polyacrylamide gel, three bands were observed with approximate molecular weights of 17, 12, 10 kD. This was clearly distinct to TGF β and were not detected using an anti-TGF β antibody on Western blotting. These data suggest that human deciduas contains a soluble, cytokine which specifically inhibits the proliferation of monocytes and may be a biological demonstration of the activity of MIC-1. Further studies are underway to clearly elucidate the activity of this extract. β

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Murine Macrophages Infected with *Leishmania major* Exhibit Reduced Nitric Oxide Production Associated with Alterations in Potassium Channel Activity

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Obligate intracellular protozoan parasites of the genus *Leishmania* can survive within mammalian macrophages through a variety of mechanisms. Infected macrophages exhibit down-regulation of a number of processes associated with activation. This can translate into functional defects, including reduced production of anti-parasitic molecules such as nitric oxide (NO). While many mechanisms of macrophage inhibition induced by *Leishmania* infection appear to be mediated through impairment of the intracellular signaling pathways required for activation, little is known about the effect of parasite invasion on host cell ion channel activity, ion homeostasis, and host cell membrane potential. Macrophages possess a number of ion channels that are important for their cytotoxic functions. Previous work in our laboratory showed that inhibition of macrophage potassium channels using a panel of pharmacological blockers resulted in reduced NO production. In this study, we report that treatment of macrophages with 4-aminopyridine (4-AP), caused alterations in potassium ion flux (measured using the potassium ion tracer 86-Rb) and membrane potential (measured using the fluorescent dye bis-oxonol) in macrophages activated with LPS and IFN- γ . The 4-AP blocks inward rectifying potassium channels, which control the membrane potential of macrophages. The infection of macrophages with *L. major* promastigotes, caused a similar reduction in NO production and alterations in potassium fluxes and membrane potential. Thus, *L. major*-induced alterations in cytotoxic responses of macrophages were comparable to those caused by the

potassium channel blockers, suggesting that potassium channels may be modulated by the parasite. This novel evasion mechanism may promote parasite survival in activated host cells. [Supported by NSERC, Canada]

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DAP12 : possible role in Fc γ R-mediated phagocytosis.

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DAP12 is a small transmembrane adaptor protein containing an ITAM motif in its cytoplasmic domain, which is associated with activating receptors. In NK cells, DAP12-associated receptors are well characterized. In contrast, function of the DAP12-associated receptors identified so far in macrophages (MDL-1, SIRP β 1, TREM-1 and TREM-2) remains unknown. In order to study the role of DAP12 in the regulation of macrophage functions, we have stably overexpressed a wild-type and a mutated epitope-tagged version of DAP12 (Flag-DAP12) in the murine macrophage cell line RAW 264.7. The mutated version (DN-Flag-DAP12) contains a mutation in its ITAM, which should act as a dominant-negative when overexpressed. Indeed, crosslinking of Flag-DAP12 but not DN-Flag-DAP12 led to TNF- α secretion. We next examined the role of DAP12 in Fc γ R-mediated phagocytosis using IgG-opsonized sheep red blood cells (IgG-SRBC). Unexpectedly, internalization of IgG-SRBC was significantly increased in DN-Flag-DAP12-overexpressing clones with respect to both controls and Flag-DAP12-overexpressing clones. This increased phagocytosis was not the consequence of an increased binding of IgG-SRBC. Since phagocytosis of latex beads was not affected by the overexpression of DN-Flag-DAP12, our results suggest that DAP12 may be implicated in the negative regulation of Fc γ R-mediated phagocytosis. Supported by the Canadian Institutes of Health Research

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Unregulated Signaling Pathways in TGF- β 1 Deficient Mice

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TGF- β plays a prominent role in regulating inflammation as evidenced by the fatal, multifocal inflammatory response in mice lacking a functional TGF- β 1 gene. The unrestricted inflammation is accompanied by constitutive activation of NF- κ B and gene expression of inflammatory cytokines, as well as IFN- γ and inducible nitric oxide synthase (iNOS), suggesting triggering of innate signaling pathways and/or failed regulation of cytokine signaling pathways. Given the preponderance of macrophages in the inflammatory infiltrates, we focused on mechanisms regulating macrophage activation in these mice. Stimulation of wild-type macrophages with IFN- γ increased iNOS gene expression which was effectively inhibited following pre-exposure to exogenous TGF- β 1. Macrophages from TGF- β 1 deficient mice also expressed increased iNOS mRNA in response to IFN- γ , but were resistant to the suppressive activity of exogenous TGF- β 1. Moreover, in vivo, exogenous TGF- β 1, whether administered as an active peptide or by gene transfer, did not reverse the inflammatory pathology in the TGF- β 1 deficient mice. Of considerable interest is the increased level of toll-like receptor TLR4, a LPS signaling molecule, in TGF- β 1 deficient mice and in mice lacking the TGF- β transcription factor Smad3. The mice are hypersensitive to bacterial LPS and NF- κ B activation, which may underlie their constitutive macrophage activation and the failure of exogenous TGF- β to reverse this already activated state. These studies suggest that innate signaling pathways may be activated and uncontrolled in the absence of TGF- β .

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The structure of LPS and lipid A influences the activation of murine macrophages via CD14-dependent or CD14- independent pathways.

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CD14 is a receptor for lipopolysaccharide (LPS, endotoxin), embedded in the outer membrane of Gram-negative bacteria. To characterize the molecular requirements of macrophage responses to LPS via CD14-dependent and CD14-independent pathways, we simultaneously compared the responses of both normal and CD14-deficient murine macrophages to structural variants of LPS including smooth and rough (Ra, Re) LPS chemotypes and different structures of lipid A. Systematic removal of carbohydrate moieties from smooth LPS lowered the CD14-mediated response but increased the CD14-independent response. Responses to chemically modified forms of lipid A demonstrate that 2 phosphates and hexaacyl fatty acid lipid A are required to evoke full activity via both pathways. However, removal of 2 phosphates from Re LPS (hexaacyl lipid A with two Kdo) strongly reduces the CD14-dependent, but not the CD14-independent response. Although the CD14-independent response increased as the LPS became more hydrophobic, in all cases the CD14 pathway was > 50 to 150,000-fold more sensitive to each form of LPS than the non-CD14 pathway, suggesting the CD14-independent response to be a non-specific pathway, most likely due to the interaction of hydrophobic structures.

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Both Platycodin D and D3 isolated from the root of *Platycodon grandiflorum* inhibit production of nitric oxide in activated RAW 264.7 cells but not TNF- α secretion.

Georgia Schuller-Levis¹, Eun Bang Lee², Chuanhua Wang¹, William Levis¹, Da Wei Lee² and Eunkyue Park¹, . Department of Immunology, NYS Institute for Basic Research in Developmental Disabilities¹ and Natural Product Research Institute, Seoul National University, Seoul, Korea². We have demonstrated that both platycodin D and D3, oligosaccharide derivatives of oleanolic acid, down regulate the inflammatory responses in orally treated rats. Since the production of PGE2 in TPA- activated rat peritoneal macrophages was inhibited by platycodin D, we examined additional major proinflammatory mediators such as of nitric oxide(NO) and tumor necrosis factor- α (TNF- α) using activated RAW 264.7 cells. RAW 264.7 cells activated with lipopolysaccharide (1 μ g/ml) and interferon- γ (50 U/ml) were treated with various doses of platycodin D and D3 for 24 hours. Supernatants were analyzed for the production of NO and TNF- α using Griess reagent and ELISA, respectively. NO was inhibited in a dose dependent manner (IC50 of platycodin \approx 15 μ M, IC50 of platycodin D3 \approx 55 μ M). Expression of inducible nitric oxide synthase (iNOS) was inhibited as measured by western blot analysis whereas expression of mRNA of iNOS was not inhibited by these compounds. In contrast to NO, secretion of TNF- α was augmented by platycodin D and D3. Expression of mRNA of TNF- α was also increased by these compounds. These data indicate that these two compounds have dichotomous regulation of NO and TNF- α , suggesting that inhibition of NO may contribute to antiinflammatory activities of these compounds.

LYMPHOCYTE ACTIVATION (106-108)

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THE NOVEL, MHC CLASS-I RELATED MOLECULES, ULBP1 AND ULBP2, BIND TO THE NKG2D/DAP10 COMPLEX, RESULTING IN FUNCTIONAL ACTIVATION OF BOTH HUMAN AND MURINE NK CELLS

J. Chalupny, P.V. Sivakumar, C. Sutherland, J. Mullberg, W. Chin, G. Jackson, M. Kubin, and D. Cosman, . Immunex Corp., Seattle, WA 98101 ULBP1, ULBP2 and ULBP3 are novel, GPI-linked, cell surface glycoproteins of the extended MHC Class I-like family. Soluble forms of the ULBPs synergize strongly with IL-12 to induce interferon gamma production from human NK cells pre-treated with IL-15 (Kubin et al, 2001, Eur.J.I., 31:1428). Soluble forms of the ULBPs alone cause increased production of the chemokine I-309 and cytokines GM-CSF, LT alpha and TNF alpha from human NK cells pre-treated with IL-15 (Cosman et al, 2001, Immunity, 14:123). ULBP1 and ULBP2 have been identified as ligands for the human

cytomegalovirus glycoprotein UL16. ULBP1, ULBP2 and ULBP3 have been identified as ligands for the receptor complex consisting of the C-type lectin, NKG2D, and the signal transducing molecule, DAP10. Cells co-transfected with human NKG2D and human DAP10 bind to ULBP1-Fc, ULBP2-Fc and ULBP3-Fc. Expression of full length forms of ULBP1, 2 or 3 on Class I+ Daudi target cells, resistant to lysis by human NK cells, restored the ability of the NK cells to lyse the targets (Cosman et al, 2001, Immunity, 14:123). This lysis could be specifically inhibited by anti-ULBP MAb or anti-human NKG2D MAb, demonstrating that this effect is due to binding of the ULBPs to NKG2D/DAP10. Cells co-transfected with murine NKG2D and human DAP10 bound to ULBP1-Fc and ULBP2-Fc but not ULBP3-Fc. Murine NK cells isolated from B6 SCID mice do not kill MHC Class I+ or MHC Class I- Daudi cells well. Co-expression of ULBP1 or ULBP2 on the Class I+ Daudi target cells rendered them susceptible to lysis by murine NK cells, whereas co-expression of ULBP3 did not. This lysis could be specifically blocked by anti-ULBP MAb. In summary, all three ULBPs are capable of transducing a dominant stimulatory signal to human NK cells, through NKG2D/DAP10, which can overcome an inhibitory signal generated by MHC Class I engagement of KIRs. ULBP1 and ULBP2, which bind to murine NKG2D, are capable of transducing a dominant stimulatory signal to murine NK cells. ULBP3, which does not bind to murine NKG2D, does not transduce a dominant stimulatory signal. The ability of the ULBPs to cross react with murine NK cells will be useful in studying the effects of these molecules in murine models of immune regulation.

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LYMPHOCYTE-PLATELET ADHESION IN IL 2 THERAPY OF PATIENTS WITH LARYNGEAL CARCINOMA

Vitkovsky Yu., Ilynykh L., Solpov A., Kuznik B. Dep. of Physiology, Chita Medical Academy, 39-A Gorky Str., Chita, 672000, Russia Earlier we have demonstrated the ability of lymphocytes carrying CD3+ and CD4+ markers to form co-aggregates with platelet spontaneously. Our findings showed that in vitro culturing of lymphocytes during four hours with interleukin (IL) 2 increased 4-fold the of rosette-forming cells in total lymphocyte pool. Stimulation of lymphocytes with IL 2 enhanced adhesive activity of CD4+ cells and induced ability of CD16+ lymphocyte to form co-aggregates with platelets. It was established that 2 hours incubation of healthy donors' heparinized whole blood in presence of IL 1beta increased 2,5 fold the number of lymphocyte-platelet co-aggregates. Other pro-inflammatory cytokines IL 8 and TNF alpha did not change ability of lymphocytes to form rosettes with platelets. However, contra-inflammatory cytokines IL 4 and IL 10 possessed inhibitory activity on lymphocyte-platelet adhesion. Adding of these cytokines in whole blood culture prevented formation of lymphocyte-platelet co-aggregates fully. Similar effect was observed for IF alpha and gamma. Our aim is to study lymphocyte-platelet adhesion and influence of IL 2 on it in patients with laryngeal carcinoma. 30 patients with II-III stages of laryngeal carcinoma were investigated. 18 patients were administered a single intravenous dose of IL 2 (1000000 U daily) in a complex therapy under immunologic control. 12 untreated patients were used as control. Lymphocyte-platelet adhesion was estimated by an original test. It was established that the number of the platelet-lymphocyte co-aggregates decreased up to 4 + 1% regardless of the stage of carcinoma. In patients who underwent only surgery or in whom surgery was combined with radiotherapy the number of platelet-lymphocyte co-aggregates remained unchanged. Administration of IL 2 to patients increased the number of lymphocyte co-aggregates regardless of the method of treatment, whereas it decreased in control patients. The lymphocyte-platelet adhesion test appeared effective for control of IL 2 administration. Cytokine administration was discontinued at the outset of the reduction of lymphocyte-platelet co-aggregate number which showed the maximal therapeutic efficacy of the medicine used. The latter is associated with both positive effect of IL 2, such as increase of the lymphocyte (T helpers, NK-cells) migration and the negative effect as well, i.e. intensified hypercoagulability. Thus, the ability of lymphocytes to form co-aggregates with platelets spontaneously decreases in patients with laryngeal carcinoma. Administration of IL 2 enhances this function and the lymphocyte-platelet adhesion test enables to control drug administration.

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Massive Activation of B Cells in Mice Following a Primary Rotavirus Infection

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Rotavirus is the leading cause of gastroenteritis in young children. However, early immunologic events following rotavirus infection are not clearly defined. We have examined some of the gut associated lymphoid tissues during a primary homologous rotavirus infection in mice to determine the sites and cellular components of the immune response. Early during infection, we observe a significant enlargement of the Peyer's patches (PP) and mesenteric lymph nodes (MLN) in infected mice, which was determined to be due to hyperplasia of lymphocytes. To determine which specific lymphocyte subtypes were responding during a rotavirus infection, lymphocytes from PP and MLN were isolated and characterized by flow cytometry using antibodies against lymphocyte subtype markers CD4, CD8, or CD19 in combination with an antibody against an early activation marker, CD69. Interestingly, only CD19/CD69 populations were significantly increased in the PP and MLN. To confirm that CD4 or CD8 T cells were not required for the presence of activated B cells, the experiment was repeated using T cell receptor knockout mice. These mice exhibited significant increases in activated B cells in the PP and MLN suggesting that the early lymphocyte responses to rotavirus infection in mice is a T cell independent B cell response. To determine if the activated B cells were capable of making rotavirus-specific antibody, fragment cultures were established using PP and MLN from both uninfected and infected mice. Upon ELISA analysis, supernatants from PP and MLN from infected mice are capable of producing rotavirus-specific IgM as early as 3 days post infection. Understanding the mechanisms of how rotavirus activates the immune system will aid in better vaccine design.

CHEMOTACTIC RESPONSES (109-110)

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Identification of alpha-Defensin-1 (HNP-1) as an Anti-Chemotactic Agent for Human Polymorphonuclear Leukocytes (PMN)

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Purpose: Regulating PMN migration into sites of injury/infection is important in the promotion as well as the resolution of the inflammatory response. Through the use of media conditioned by TNF- α -stimulated PMN (CM-TNF), we have shown that PMN have the ability to suppress the migration of other PMN to several chemoattractants. In this respect, the purpose of this study was to identify and characterize the agent(s) in CM-TNF which mediate this anti-chemotactic activity. Methods: CM-TNF was fractionated by HPLC. Fractions were assayed for bioactivity utilizing a migration assay in which PMN were resuspended in the fractions and allowed to migrate across ECM-coated Transwell filters. Bioactive fractions were analyzed by MALDI-TOF. Recombinant peptides were utilized to demonstrate anti-chemotactic agent of the identified fraction. Results: Two fractions obtained by HPLC fractionation of CM-TNF contained anti-chemotactic activity. MALDI-TOF analysis of one fraction identified the protein as HNP-1 (human neutrophil protein 1) or α -defensin 1. Recombinant HNP-1 demonstrated a dose-dependent suppression of migration to fMLP. However, consistent with the inability of CM-TNF to suppress migration to IL-8, recombinant HNP-1 did not suppress migration to this chemoattractant. Conclusions: We demonstrate that one of the factors released by TNF- α -stimulated PMN that suppresses PMN migration is the granule protein, HNP-1. This data expands upon the role of this protein in the inflammatory process: a bactericidal agent, a chemokine for a select population of inflammatory cells, and now, an anti-chemotactic agent for PMN.

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IDENTIFICATION OF A MODIFIED FORM OF THE VITAMIN D BINDING PROTEIN (DBP) THAT FUNCTIONS AS A CO-CHEMOTACTIC FACTOR FOR C5a

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The vitamin D binding protein (DBP), also known as Gc-globulin, is a multifunctional plasma protein that can enhance leukocyte chemotaxis to complement peptide C5a. However, the mechanisms underlying this cellular response are not known. In this study we investigated if DBP needs to be modified in order to function as a co chemotactic factor for C5a. Peripheral blood neutrophils (PMN) and undifferentiated U937 cells transfected with the C5a receptor (U937-C5aR) were used to measure chemotaxis. Purified DBP, plasma or serum (used as DBP sources) all induce PMN to display an enhanced chemotactic response to C5a (co-chemotaxis). In contrast, U937-C5aR only show co-chemotactic movement to serum-derived DBP. Moreover, U937-C5aR show significantly greater movement to zymosan-activated serum than activated plasma. The results show a clear difference in co-chemotactic capacity of DBP in serum versus plasma, suggesting that platelets and/or the clotting cascade converts DBP into the active co-chemotactic form. However, there is no alteration in the mol wt of DBP in serum versus plasma, and the serum-derived modification is not affected by a complete protease inhibitor cocktail, largely discounting proteolytic cleavage as the modifying mechanism. In addition, we have observed that the active form of vitamin D (1,25-OH D3) suppresses co-chemotactic activity, perhaps by activating alkaline phosphatase. These results suggest that a reversible modification of DBP, possibly phosphorylation, converts the protein into an active chemotactic cofactor for C5a.

SECRETION/PHAGOCYTOSIS (111-112)

111

Inhibition of the chemiluminescence of human polymorphonuclear leukocytes and carrageenan-induced rat's paw oedema by a newly synthesized 4-amino uracil derivative

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Compound I (4-(N-dimethylaminoethyl) aminouracil) (12.5 μ 500mg/ml) dose-dependently inhibited the chemiluminescence (CL) response of isolated human polymorphonuclear leukocytes (PMNs) stimulated by phorbol myristate acetate (PMA) or opsonized zymosan (OPZ). The compound (12.5 μ 200mg/ml), also, inhibited the superoxide production by human PMNs. It did not interfere with phagocytosis of opsonized yeast by PMNs at doses of 12.5 μ 500mg/ml. Viability of isolated human PMNs was not significantly affected by various concentrations of the compound (12.5 μ 500mg/ml). The compound (15, 30, 60 and 120mg/kg, i.p.) was able to inhibit the carrageenan-induced rat's paw oedema. These results indicate that the aminouracil inhibits CL of isolated human PMNs *in vitro* and the carrageenan-induced inflammation *in vivo*.

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Macrophage Migration Inhibitory Factor (MIF) Interacts with the Vesicle-tethering Protein p115: A Possible Route for the Secretion of Leaderless MIF.

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Macrophage migration inhibitory factor (MIF) has emerged to be an important regulator of the innate and acquired immune response and has the unique capacity to counter-regulate the inhibitory effects of glucocorticoids on pro-inflammatory cytokine expression. MIF is expressed in several cell types, and secretion follows specific stimulation of monocytes/macrophages, T

lymphocytes, eosinophils, epithelial cells, and certain endocrine cells. MIF protein has been visualized within the secretory vesicles of the anterior pituitary gland, however the gene product for MIF does not contain a classical leader sequence indicating that the protein likely follows a non-classical secretory pathway. To identify possible mechanisms for MIF secretion, we searched for interacting chaperone proteins in a pituitary cDNA library using the Cyto-trap yeast two-hybrid system. A primary screen yielded over 90 putative targets that were reduced to 33 candidates upon elimination of temperature sensitive mutants. Secondary screening reduced these lead targets to 15 clones, 14 of which were MIF, and finally revealed a 260 amino acid COOH-terminal fragment of the vesicle-tethering protein p115 as an interacting partner of MIF. This interaction was verified in the Cyto-trap system using non-MIF "bait" constructs and further validated by *in vitro* co-precipitation of full-length p115. p115 has been shown to be critical for the trafficking of vesicles between the ER and the Golgi and for the binding of transcytotic vesicles to the plasma membrane. p115 may play an essential role in the secretion of MIF by recruiting the protein to the Golgi apparatus, or by concentrating it within the apical vesicles of the plasma membrane.

NADPH OXIDASE (113-114)

113

Cloning of the Rabbit Leukocyte NADPH Oxidase

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The NADPH oxidase plays an important role in both immune and non-immune cell functions. Because rabbits represent an established model for studying a number of important disease processes that involve NADPH oxidase activity, we carried out studies to clone and sequence all five rabbit leukocyte NADPH oxidase genes. Comparison of the rabbit sequences with those of other species showed that, with the exception of p67phox, the rabbit phox proteins were highly conserved. In contrast, rabbit p67phox had a very divergent C-terminus and was 17 amino acids longer than any other known p67phox homolog. This was surprising, given the high degree of conservation among all of the phox proteins previously sequenced. To evaluate the functional consequences of this difference, wild-type rabbit p67phox and a mutated rabbit p67phox missing the C-terminal 17 amino acids were expressed and analyzed in a cell-free assay. Our results show that both the full-length and truncated rabbit p67phox proteins were able to support oxidase activity, although the truncated form reproducibly supported a higher level of activity than full-length p67phox. These studies contribute to our understanding of the nature of the leukocyte NADPH oxidase in different species and will be valuable in future research using the rabbit model.

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REGULATION OF THE NADPH OXIDASE OF NEUTROPHILS BY RAC2 AND RHOGDI

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The NADPH oxidase of neutrophils generates superoxide anion that plays a role in host defense and inflammation. We observed that under our standard conditions of the cell-free NADPH oxidase system (3 nM cyt b, 10 nM prenylated Rac2, 50 nM p67, and 50 nM p47) superoxide production can occur in the absence of SDS. The rate of superoxide production was 50% of that observed in the presence of SDS (90 μ M). Increasing the concentration of Rac2 and p67 by 3-5 fold in the absence of SDS raised superoxide levels to that observed in the presence of SDS under standard conditions. Prenylated Rac2 was active only in the GTP-bound state. We examined the effect of RhoGDI on Rac regulation of the NADPH oxidase under these conditions. RhoGDI inhibited superoxide production in the absence of SDS. Titration of SDS back into this system, relieved the inhibition caused by GDI. We examined the role of Rac2 (standard conditions and no GDI) in both electron

transfer reactions of the NADPH oxidase. We observed that while both Rac2 and p67 were required for the first electron transfer step from NADPH to FAD (as measured by INT reduction), they did not need to interact with each other as shown by using mutants of Rac and p67. However, their interaction was required in the second step of electron transfer from FAD to cyt b heme and oxygen. Using a fluorescent analog of GTP bound to Rac2, we observed a physical interaction of Rac2 with cyt b. The domains of Rac that may be involved in this interaction are currently being investigated. These data establish a new molecular model for NADPH oxidase regulation by Rac in neutrophils.

EFFECTOR MECHANISMS OF PHAGOCYTES (115-118)

115

Differential Nitric Oxide Production by Immune Cells of Chickens

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Nitric Oxide (NO) is one of the end products of the metabolism of L-arginine to L-citrulline by nitric oxide synthase. It is a free radical with an unpaired electron, which reacts rapidly with molecular oxygen and oxygen radicals. It has both cytotoxic effects during inflammatory responses and regulatory effects as a component of physiological signaling transduction cascades. The cytotoxic effects require large amounts of NO and are an essential mechanism for control of invading microbes. The regulatory effects require much lower concentrations of NO. We quantified the production of nitrite, a stable metabolite of the NO pathway, in chicken macrophages, monocytes and heterophils after stimulation by IFN γ , LPS and bacteria. Our results demonstrate that there is a differential activation of the NO pathway in each of these cell types. The tissue macrophage (HD-11) produced an average of 2.7 fold more nitrite (μ M) in comparison to peripheral blood monocytes and 27.7 fold more than peripheral blood heterophils. The minimal NO production by heterophils suggest that its primary purpose is regulatory not cytotoxic. Although heterophils have previously been demonstrated to be a major mechanism by which the innate immune system defends against microbial invasion, it would appear that control is accomplished via mechanisms other than NO production, such as oxidative burst and phagocytosis.

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The Consensus Transport Signature Sequence of Nramp1 is Required for Iron Transport.

Christina M. Bishop, Donald E. Kuhn, William P. Lafuse, and Bruce S. Zwillig. Departments of Microbiology and Molecular Virology, Immunology, and Medical Genetics, The Ohio State University, Columbus, OH 43210. Innate resistance to mycobacterial growth is mediated in mice by a protein Nramp1 (Solute Carrier Family SLC11A1) which is expressed in cells of the myeloid lineage. Nramp1 is an integral membrane protein containing 10-12 transmembrane segments and a consensus transport signature sequence. Previous studies from this laboratory have shown that Nramp1 transports iron into *M. avium* containing phagosomes where the iron serves as a catalyst for the generation of hydroxyl radicals. We have initiated site directed mutagenesis experiments to study the regulation of Nramp1 iron transport. In the current study we created mutations in the transport signature sequence TMT-4X-CQ-4X-GF in which the conserved sequences TMT, CQ, and GF were changed to alanines. A fourth mutant was also created in which the transport signature sequence was deleted. Each mutant and wild-type Nramp1 was expressed in the pcDNA3.1/myc-His expression vector and stably transfected into RAW264.7 cells. Iron uptake by *M. avium* containing phagosomes was examined by *M. avium* infection of transfected cell lines that had been cultured in ⁵⁵Fe-citrate for 16 hours to radiolabel the iron pools. After two hours of

infection, phagosomes were isolated and radiolabeled iron uptake by the phagosomes was determined. Each of the mutations dramatically inhibited iron uptake by Nramp1 but did not affect the expression of Nramp1 in the phagosome. The kinetics of iron transport by isolated phagosomes is being evaluated. We are also examining the effect of the mutations on resistance to mycobacterial growth. Our results suggest that the transport signature sequence is critical for the iron transport function of Nramp1. (This work was supported by grants DK-57667, AI-42901, and HL-59795 from NIH to B.S.Z. and W.P.L.).

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Increased Resistance to Mycobacterial Growth by Alpha-2 Adrenergic Stimulation of Macrophages Requires both Nitric Oxide and Superoxide.

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Our laboratory has previously shown that epinephrine and norepinephrine stimulated resident peritoneal macrophages to resist the growth of *Mycobacterium avium* by binding to the α -2 adrenergic receptor. In the current study, we examined the mechanism by which clonidine, an α -2 agonist, stimulated the RAW264.7 macrophage cell line to inhibit the growth of *M. avium*. Clonidine treatment increased resistance of macrophages to mycobacterial growth in a dose dependent manner. This inhibition of mycobacterial growth was restricted to α -2 adrenergic stimulation since treatment of the cells with α -1, β -1, and β -2 agonists were without effect. The effect of clonidine on resistance of macrophages to mycobacterial growth was blocked with a Gi specific antagonist but not with a Gs specific antagonist. The increased resistance to mycobacterial growth resulting from clonidine stimulation was prevented by treating macrophages with inhibitors of nitric oxide synthase (L-NMMA and aminoguanidine) and with DPI, an inhibitor of NADPH oxidase and MnTBAP, a SOD mimetic which acts a scavenger of superoxide. Since NO and superoxide react to form peroxynitrite, we also treated macrophages with FETPPS (a specific scavenger of peroxynitrite). FETPPS inhibited the antimicrobial activity of clonidine stimulated macrophages. Although we found iNOS to be required for the increased resistance stimulated by clonidine, the α -2 agonist did not affect the level of NO produced by the macrophage. (This work was supported by grants AI-42901, DK-57667, and HL-59795 from NIH to B.S.Z. and W.P.L.)

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Role for bystander cell participation and cognate cytokine production for rapid induction of human CD8+ T cell effector function.

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Rapid induction of effector responses require the concerted interaction of antigen-specific T cell with its cognate peptide-bearing APC (antigen-presenting cells). While numerous studies have delineated the cellular and humoral immune requirements for the maintenance and expansion of antigen-specific CD8+ T cells, the role of these factors during the acute effector phase is not as well characterized. In this study, the requirement of these factors for optimal induction of antigen-specific CD8+ T cell responses was evaluated by directly quantitating CD8+ T cell IFN- γ response by ELISPOT assays and intracellular cytokine staining and flow cytometry. Additionally, to evaluate the biological effects of IFN- γ -mediated effector responses, we developed a real-time bioassay specific for detecting IFN- γ function based upon the induction of the chemokine MIG (monokine induced by gamma interferon) in APC. Using peptides containing well-defined CD8+ T cell epitopes from FLU, CMV, and EBV antigens, our studies demonstrate that peptide-specific induction of MIG expression is strictly dependent upon IFN- γ production from antigen-specific CD8+ T cells. This model allowed us to delineate the requirement of cellular and humoral (cytokine) factors at the level of induction of CD8+ T cell effector response (IFN- γ production) and to assess a role for these factors for optimal induction of antigen-specific IFN- γ -mediated immune responses as determined by induction of MIG expression.

CYTOKINES SUPERFAMILY MEMBERS (119-122)

119

Generation of Antagonists by Amino Acid Replacement in the D-Helix of Human IL-21

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Interleukin 21 (IL-21) is a recently described four-helix-bundle cytokine that binds to the heterodimeric receptor complex of IL-21R and IL-2 gamma common (IL-2 γ c). This and its homology, place IL-21 in the IL-2 cytokine family, which includes IL-2, IL-4, IL-7, IL-9 and IL-15. In vitro analysis demonstrates a role for IL-21 in NK cell proliferation and maturation of bone marrow, proliferation of B-cells when co-stimulated with anti-CD40, and proliferation of T-cells when co-stimulated with anti-CD3. It has previously been shown that with cytokines in the IL-2 family, amino acid substitutions within the D-helix can inhibit cellular signaling without changing binding affinity to the receptor. This has been reported to be due to alteration of the interaction of the cytokine with IL-2 γ c. Here we report the identification of mutations within the D-helix of IL-21 that eliminate its biological activity, as measured by proliferation of BaF3 cells expressing human IL-21R. Two IL-21 D-helix mutations completely block receptor activation, yet do not alter the affinity of binding to the IL-21 receptor complex.

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Cytokine-synthesizing activity of erythroid nuclear cells of human bone marrow.

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It is known that practically all cells of bone marrow (BM) produce haemo-immunoregulatory molecules. In the other hand the cytokine-synthesizing activity of the erythroid nuclear cells (ENC) of human BM practically was not studied. As shown earlier in embryonal period the ENC of fetal liver produce a number of haemo-immunoregulatory cytokines. Purpose of our investigation was to study the ability of human BM ENC to produce various cytokines. Erythroid cells were isolated by positive and negative selection (ENC made 97% of total population). ENC of human BM (10^6 cells/ml) were cultivated 24 hours. Conditional media of erythroid cells was isolated and concentration of cytokines measured by electrochemiluminescence method by using poly- and monoclonal antibodies. We have found that BM ENC of healthy donors produce cytokines: IL-1, IL-2, IL-4, IL-6, IL-10, TNF- α , IFN- γ and TGF- β 1. We have obtained the conditional media of ENC population derived from CFU-E grown on the methylcellulose (Stem Cells, MethoCult GFH4434, ENC made 100% of total population). Measurement of a cytokine concentrations in ENC conditional media shown cytokine production by BM ENC completely proves to be true by production same cytokines ENC allocated from erythroid colonies. Level of cytokine production by BM ENC is comparable with a level of production of same cytokines by mitogen-activated peripheral blood mononuclear cells. Thus, BM ENC produce a number of cytokines that can regulate haemo- and immunopoiesis in human bone marrow.

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Presence of a Nuclear Export Signal-like Sequence within the Structure of the Human Interleukin-1 α Precursor

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IL-1 α is primarily translated as a 33 kDa molecule (IL-1 α 1-271) and then post-translationally processed into a 17 kDa molecule (IL-1 α 119-271) by calpain. The precursor region of IL-1 α (IL-1 α 1-118) contains a nuclear

localization signal (KVLKKRRL, residues 79-86). To determine the intracellular localization of IL-1 α , we used chimeric genes constructed by fusion of either IL-1 α 1-118 or IL-1 α 1-271 with β -galactosidase (β -gal). IL-1 α 1-118 was localized in the nucleus, while IL-1 α 1-271 was localized in both the nucleus and the cytoplasm, suggesting the presence of a nuclear export signal. To delineate the region responsible for this nuclear export, we constructed various mutants with deletion of a part of the mature region from the C terminus. We then examined which region affects the intracellular localization of IL-1 α 1-271. IL-1 α with deletion of 70 residues from the C terminus (IL-1 α 1-201) was localized in both the nucleus and the cytoplasm, while IL-1 α 1-167 was localized only in the nucleus. IL-1 α with internal deletion of this region (IL-1 α 1-167/202-271) was localized in the nucleus, while this region (IL-1 α 168-201) fused to β -gal was localized only in the cytoplasm. Thus, this study suggests the presence of a nuclear export signal-like sequence in the region comprising residues 168-201, and that intracellular localization of IL-1 α may be regulated not only by a nuclear localization signal but also by a nuclear export signal-like sequence.

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Interleukin 18 is involved in Acetaminophen-induced liver injury in a Fas/Fas ligand independent mechanism

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Introduction: IL-18 is a novel pro-inflammatory cytokine that participates in hepatocyte apoptosis in mice after treatment with a variety of hepatotoxins. The normal apoptotic pathway commences with Fas/Fas ligand interaction. The role of IL-18 in APAP-induced hepatic injury has not been previously reported. **Objective:** To explore the role of IL-18 in APAP-induced hepatic injury and determine if this mechanism is Fas/Fas ligand mediated. **Methods:** C57BL/6 female mice (N=10) were injected intraperitoneally (i.p.) with 700 mg/kg APAP (pH=10.5). Control mice (N=10) received an equal volume of saline (pH=10.5) by the same route. Blood and liver were collected for ALT and IL-18 determination at 1, 2, 4, 8, 24 hours following APAP injection. A second group (N=25) was injected i.p. with neutralizing antibodies to IL-18 one hour prior to APAP injection. ALT and IL-18 levels were measured at 8 hours following APAP injection. Control mice (N=25) received normal rabbit serum one hour prior to APAP injection. Ten Fas deficient mice (Lpr/Lpr) were also injected i.p. with APAP to explore Fas/Fas Ligand participation. **Results:** IL-18 was detected in the serum and liver one hour following APAP injection and reached peak elevation after 8 hours. Anti-IL18 antibodies significantly reduced the injury: mean serum ALT (\pm SEM) was 523 ± 85.6 U/L compared to, 1653 ± 210 U/L for controls ($p < 0.0001$). Mean serum ALT for Lpr/Lpr mice was not different from wild type mice. **Conclusion:** In this model of acute liver failure, IL-18 participates in APAP-induced hepatotoxicity, but IL-18 antibodies do not completely prevent hepatic injury. The mechanism by which IL-18 is involved in APAP hepatotoxicity seems to be Fas independent.

TNF-RELATED CYTOKINES AND RECEPTORS (123-127)

123

Inhibition of Experimental Autoimmune Encephalomyelitis by an Antibody Specific for Mouse CD30L

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CD30 and CD30L are members of the TNF receptor and TNF ligand superfamilies, respectively. Molecular interactions among the members of these families have been shown to have important immune regulatory functions. However, the biological significance of interactions between CD30 and its ligand is not well defined. We generated a rat IgG2a monoclonal

antibody specific for mouse CD30L and used it to explore the role of CD30/CD30L interactions in mice. The anti-CD30L mAb specifically blocks the binding of mouse CD30 and CD30L and stains activated, but not resting T cells. When used in vitro, anti-CD30L mAb had no inhibitory effect on anti-CD3 or antigen-specific T cell proliferation or cytokine production. However, when used in vivo, anti-CD30L mAb exhibited potent inhibitory effects in several mouse models of autoimmunity. Prophylactic treatment with anti-CD30L inhibited IgE production in a CD40L-independent model of GVHD and inhibited the development of collagen-induced arthritis and experimental allergic encephalomyelitis (EAE). In the MOG35-55 induced model of EAE in C57BL/6 mice, treatment with anti-CD30L after immunization, but before the onset of clinical signs, reduced the incidence of clinical and histological signs of EAE. Among the anti-CD30L treated mice that developed clinical signs of EAE, the onset of disease was delayed and the weight loss associated with disease was reduced relative to controls. Anti-CD30L treatment does not appear to inhibit T cell priming, skew from a Th1 to a Th2 type response, deplete or block in vivo expansion of activated T cells. Although the mechanism(s) by which anti-CD30L exerts its immunomodulatory effects in vivo are not yet clear, these results suggest that antagonists of CD30/CD30L interactions may have utility for the treatment of inflammatory or autoimmune diseases.

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Lymphotoxin (LT) function in vivo as dissected by inactivation of LT complex in B cells.

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Signaling by membrane LT β / α complex plays a critical role in development and maintenance of the secondary lymphoid organs. To address the role of LT β expressed by B cells, the LT β gene was deleted specifically in B cells (99% of deletion), which resulted from crossing "floxed" LT β mice with CD19Cre knock-in mice (B-LT β KO). LT β inactivation in B cells resulted in the lack of polarized follicles, disruption of marginal zone, defective germinal centers in the spleen and impaired primary and secondary IgG responses to SRBC. In B-LT β KO mice, in contrast to LT β KO mice, follicular dendritic cell (FDC) clusters were present (although largely reduced) in the spleen, while DC and NK cell numbers were normal in the spleen. SCID mice injected with B cells from B-LT β KO failed to develop FDC in the spleen, suggesting that T cells contribute to FDC generation. In contrast to the spleen, FDC clusters were normal in mesenteric lymph nodes (MLN) and Peyer's patches (PP) of B-LT β KO mice, showing that requirements for maturation of FDC in MLN, PP, and spleen are different. Thus, B-LT β KO mice demonstrate a partial phenotype compared to mice with complete LT β deficiency, implying that other cell types contribute to LT β -mediated effects in vivo. This is currently being addressed by the analysis of mice with T-LT β and B-TNF deficiencies. (Research at NCI was supported by NIH Contract NO1-CO-56000 and grant from IARC).

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Dual function of TNF as regulator of myelopoiesis in long-term bone marrow cultures.

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TNF is a pleiotropic cytokine, which exerts a wide range of effects on the hematopoietic system. It has been characterized as a bi-directional regulator of hematopoiesis, with its main biological activity attributed to inhibition of CSF-induced proliferation of hematopoietic progenitors in vitro. In order to assess the physiological relevance of TNF in hematopoiesis, we established long-term bone marrow cultures (LTBMC) from TNF $^{-/-}$ mice. The total number of non-adherent (NA) cells in TNF $^{-/-}$ LTBMC was significantly

increased after 5 weeks compared to WT controls. This increased cellularity was not due to a decreased apoptotic activity of TNF-/- cells, but rather due to a significant increase in the number of progenitors in TNF-/- LTBM. Morphological analysis of NA cells revealed fewer differentiated cells in TNF-/- LTBM than in WT LTBM. To determine whether there was an increase in the number of progenitors in the bone marrow of TNF-/- mice, we purified a population of Lin-cKit+Sca1+ cells enriched for pluripotent hematopoietic cells and a population of Lin-cKit+Sca1- cells enriched for more committed progenitors. We observed a significant increase in the number of GM-CSF-responsive progenitor cells in bone marrow of TNF-/- mice compared to WT controls. Collectively, our data suggest that TNF may affect myelopoiesis in vivo by regulating the number of CFU-GM and their differentiation. (Research at NCI supported by Contract#NO1-CO-56000)

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Biological functions of Tumor Necrosis Factor and Lymphotoxin in vivo assessed using a novel panel of knockout mice.

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Tumor Necrosis Factor and Lymphotoxin are closely related cytokines with both distinct and overlapping activities in vitro and in vivo. In order to define more precisely the cell types responsible for these activities in vivo, we collaborated with laboratories of L. Tessarollo and C. Stewart (NCI Frederick) to generate mice in which each of the three genes of the TNF/LT locus has been tagged for subsequent conditional Cre-mediated deletion in vivo. Using Cre transgenic mice from the laboratories of K. Rajewsky, R. Rickert, J. Takeda and I. Forster we then generated 6 strains of mice in which TNF or LT expression was specifically disrupted either in B, or in T cells, or in macrophages/neutrophils. Additionally, addressing the concern of possible neo-mediated alteration of gene expression in the compact TNF/LT locus in previously generated conventional knock-outs, we produced mice with neo-free deletions of each of the three genes in the locus. The comparative analysis of peripheral lymphoid tissues and evaluation of immune and host defense functions in all these strains will be discussed. (Research at NCI was supported by NIH Contract NO1-CO-56000)

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Modulation of Death Receptors by Adenovirus E3 Proteins Requires Intracellular-Trafficking Motifs

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Adenovirus encodes multiple gene products that regulate proapoptotic cellular responses to viral infection mediated by the innate and adaptive immune systems. The E3-10.4K and 14.5K gene products exist in a complex and are known to modulate the death receptor Fas. Recently, we reported that the viral E3 protein 6.7K works in concert with 10.4K and 14.5K to modulate the two death receptors for the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Expression of these E3 proteins results in reduced cell surface levels of Fas, TRAIL Receptor-1 and 2 and inhibits ligand-mediated killing of HT-29 cells. The 10.4K, 14.5K and 6.7K gene products contain motifs thought to be important for protein trafficking. In this study, we demonstrate that mutation of any of three motifs in 10.4K or one of two motifs in 14.5K results in complete abrogation of receptor downregulation. Mutation of the second motif in 14.5K or the single motif in 6.7K does not affect this activity. Mutants that fail to alter receptor levels are also unable to inhibit Fas- or TRAIL-mediated killing. E3 complex formation is not affected by any of the mutants. Our results suggest that correct intracellular trafficking is essential for the ability of 10.4K and 14.5K subunits to regulate the activity of Fas and TRAIL receptors. This work was supported in part by grants AI33068, CA69381, AI48073 and AI10414 and ACS grant PF0100401.

GENOMICS OF CYTOKINE GENE EXPRESSION (128)

128

Generation of diversity in the innate immune system. Macrophage heterogeneity arises from gene-autonomous transcriptional probability of individual inducible genes.

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Microbial products such as lipopolysaccharide (LPS) stimulate macrophages to produce a wide diversity of inducible gene products needed for immediate host defense and priming of an appropriate acquired immune response. The murine macrophage cell line, RAW264 has been extensively used to study macrophage function and gene expression. We, and others, have found that sub-clones of this cell line have considerable divergence in terms of LPS-inducible gene expression. In this study, we have used cDNA microarrays to investigate this phenomenon on a genome wide scale. Even archetypal target genes such as TNF- α were not induced in all subclones, and there was no absolute correlation between expression of pairs of genes. Nevertheless, the array analysis revealed clusters of genes that were more likely to be co-expressed. RAW264 cells stably transfected with luciferase reporter genes driven by LPS-responsive promoters revealed the same kind of clonal heterogeneity. The results indicate that each LPS-inducible gene has its own inherent probability of activation in response to LPS. If the underlying mechanism is probabilistic then each committed macrophage progenitor is unique and the innate immune system can present an infinitely complex defence network to resist an invading pathogen.

GENETICS OF CYTOKINE GENES/DISEASE LINKAGE (129-130)

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Interleukin-1 complex genotype is associated with susceptibility to asthma but only in males

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Asthma is a multifactorial disease in which dozens of genes and environmental factors influence morbidity. One of the major components in its pathogenesis is inflammation. The intensity of inflammation is, at least to a significant extent, genetically regulated, i.e. the genes of the pro- and antiinflammatory cytokines are polymorphic and several of these polymorphisms have been shown to be associated either with severity of or susceptibility to many inflammatory diseases. There are also clear gender-dependent differences in the prevalence and clinical picture of several inflammatory diseases. In asthma both the prevalence and incidence are higher in females. To examine the role of the genetic variation of inflammation in asthma we analysed the polymorphism of the IL-1 beta gene in a cohort of asthma patients (n=245, mean age 58 years, range 31-84 years, mean duration of asthma 11 years) and 405 controls. The genotyping of the single nucleotide exchange at the position -511 was performed with PCR-RFLP. The results obtained demonstrated that there were no differences in the allele frequencies. However, in males the genotype distribution differed significantly between asthmatics and controls (p=0.03, 3x2 chi-square test) and this was mainly due to the decreased number of 1.2 heterozygotes in the patient group (p=0.01). To establish whether the effect of the IL-1 genotype is stronger in an asthma subgroup, the male patients and controls were divided into groups using the serum IgE levels as criterion (> 100 IU/ml vs. < 100 IU/ml). The genotype distribution between cases and controls was significantly different only in males with lower IgE levels (p=0.009). The odds ratio of the 1.2 heterozygote males was in this case 0.37 (95 percent confidence intervals 0.19-0.71). In summary, these data

demonstrate that the genetic background of asthma differs between males and females. In the case of males the effect of the IL-1 beta locus is clear, while in females it has no effect. The odds ratios observed in males are of the same magnitude as with other asthma susceptibility genes. However, the findings described here make a significant contribution to the total asthma prevalence due to the high number of the functional genotypes (i.e. 50/50 IL-1beta homozygote/heterozygote ratio).

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Contrasting Evolution of the Human Leukocyte N-formylpeptide Receptor Subtypes FPR and FPRL1R

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N-formylpeptides are phagocyte chemoattractants that act by binding to two structurally related receptors, FPR (formylpeptide receptor) and FPRL1R (FPR-like-1 receptor), which are encoded by the human genes FPR1 and FPRL1. Single nucleotide polymorphisms (SNPs) in the FPR coding region have been reported and two have been associated with the disease Juvenile Periodontitis; however, their frequency and linkage relationships are unknown. Here we systematically analyzed polymorphism in the open reading frames of FPR1 and FPRL1 by direct sequencing of cloned alleles from random blood donors from North America. For FPR1 we detected 5 non-synonymous SNPs and 2 synonymous SNPs in a sample of 26 chromosomes one each from 17 Caucasian and 9 Black random blood donors. Although all 5 non-synonymous SNPs were common in Caucasians, Blacks, and Asians, notable differences in allele frequency were found for each SNP in the different racial groups, suggesting differential selective pressures. We found that the FPR1 polymorphisms are linked in 15 common haplotypes. No polymorphisms were detected in FPRL1 after sampling 44 chromosomes from 36 random blood donors from the same three racial groups. Thus FPR1 and FPRL1, though they originated from a common gene, appear to have undergone markedly different evolutionary events.

CYTOKINE GENE REGULATION (131-141)

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PLEIOTROPIC EFFECTS OF IL-2 AT THE MOLECULAR LEVEL

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IL-2 induces the stimulation of inflammatory and immune reactions and the apoptosis of antigen-activated cells. However the molecular basis of these pleiotropic effects is largely unknown. To study further the molecular basis of IL-2 function, we used a cDNA subtraction approach using a cell line grown in IL-2 or IL-4. From the corresponding library, a large number of relevant non-redundant sequences were characterized. The in vivo expression of these genes was analyzed in spleen and lymph node cells of IL-2-deficient and MRL/lpr mice, which both have high numbers of activated cells but the latter have intact IL-2 expression. IL-2 increased the expression of cytoskeleton proteins (α -tubulin,...), oncogene-regulating proteins (CTCF, JIF-1,...), and transcription factors (E2F-4, CREB, zhx-1,...). IL-2 also regulated the expression of genes coding for multifunctional proteins, e.g. β α -catenin and nucleolin. We also demonstrate in vitro and in vivo that IL-2 controls the expression of genes of the TNF family. Strikingly, the expression of TNF- α , TNF- β , LT- β , TNFR1 and TNFR2 mRNA levels are extremely low in the spleens of IL-2-/- animals. A similar analysis of thymocytes from IL-2-/- and IL-2+/- mice demonstrated the same expression patterns of all the studied sequences in these two strains. Taken together, our data provide additional evidence for the pleiotropic action of IL-2 in the secondary lymphoid organs and IL-2-independence of molecular processes involved in thymocyte differentiation.

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THE -174 POLYMORPHISM OF IL-6 ALTERS BINDING OF A HELA TRANSCRIPTION FACTOR AND FORMS FUNCTIONALLY SIGNIFICANT ALLELIC ASSOCIATIONS WITH OTHER 5'-FLANKING REGION POLYMORPHISMS.

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We have previously reported that the IL-6 5'-flanking region polymorphism G-174 is a higher expresser of IL-6 than C-174 in vitro and in vivo. The potential influence of two other polymorphisms (G/A-598 and G/C-572) has been investigated by genotyping and transfection studies, and the functional effects of -174 further investigated using electrophoretic mobility shift assays (EMSA). In 383 healthy Caucasian controls G-598 showed strong allelic association with G-174, and A-598 with C-174 (linkage disequilibrium =0.704). C-572 (rare allele) showed weak allelic association with G-174 (LD=0.195). In HeLa and 3T3 cells, transfection of the common haplotype G-598G-572G-174 was associated with significantly higher IL-6 transcription than the other common haplotype A-598G-572C-174 ($p=0.049$) when induced with IL-1, consistent with -174 allelic effects. There was no independent functional effect of the -598 polymorphism when G-598G-572G-174 and A-598G-572G-174 were compared. -572 had independent functional effect with G-598G-572G-174 being a higher expresser than G-598G-572G-174 ($p=0.005$). These effects appear to be cell specific, as they were not seen in Huh 7 cells. EMSA studies revealed a HeLa nuclear factor to bind more strongly to C-174 than G-174. C-174 required higher concentrations of cold probe than G-174 to compete the band out (approx. 2-fold difference), and C-174 cold probe was a stronger competitor than G-174 cold probe. This factor bound a site around -174 more strongly in unstimulated than IL-1 induced extracts suggesting that it may be an inhibitory factor. In conclusion, there are functionally significant allelic associations between -174, -572 and -598 polymorphisms of IL-6, but only -174 and -572 exert independent effects on IL-6 regulation. The -174 allelic effects may be explained by the differential binding of an inhibitory transcription factor at this site.

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NF κ B-mediated TLR2 Gene Expression Requires the Transcription Factor Sp1

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Toll-like receptor 2 plays an essential role in initiating the cellular response to various bacterial components. TLR2 gene expression is rapidly induced following infection with *Mycobacterium avium*. We have previously identified two NF κ B sites within the 326-bp proximal TLR2 promoter to be necessary for the induction of transcription of TLR2 promoter. However, in the present study we found that the NF κ B sequence by itself was not able to confer *M. avium*-reactivity upon a heterologous promoter, indicating the requirement of other elements. By contrast, two tandem-arrayed Sp1 sites located at -145 to -112 relative to the first transcription start site were important for TLR2 transcription because mutating or eliminating these two sites markedly reduced the *M. avium* sensitivity. Electrophoretic mobility shift assays demonstrated that the Sp1 sites bound Sp1 and Sp3, although the level of binding activity did not vary with infection. Further studies showed that overexpression of NF κ B p65 caused a dramatic increase in transcription from an intact TLR2 promoter while it caused only a partial increase in promoter activity when cotransfected with the TLR2 promoter with one of the Sp1 sites mutated. Thus we hypothesized that NF κ B may cooperate with Sp1 to activate TLR2 transcription upon infection. This was confirmed by the observation that Sp1 and NF κ B were the minimum mammalian transcription factors required for effective TLR2 transcriptional activity when transfected into *Drosophila* S2 cells, which lack endogenous Sp1 and NF κ B factors.

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Accelerated wound healing in TNF receptor p55 deficient mice with reduced leukocyte infiltration

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We examined the interaction between TNF receptor p55 (TNF-Rp55)-mediated signals and extracellular matrix (ECM) production in wound healing. Materials and methods: We prepared excisional skin wounds in BALB/c (WT) and TNF-Rp55-deficient mice (TNF-Rp55^{-/-}). Macroscopic wound closure, leukocyte infiltration, hydroxyproline (HP) contents, and the gene expression of MMPs were evaluated. Results and discussion: Macroscopically, wound closure was significantly accelerated in TNF-Rp55^{-/-} compared with WT. Immunohistochemically, neutrophil and macrophage infiltration was the most evident at 1 day and 6 days after the injury in both mice, respectively. However, the number of neutrophil and macrophage was significantly reduced in TNF-Rp55^{-/-}, compared with WT. The contents of HP significantly increased at 3 and 6 days in TNF-Rp55^{-/-}, compared with WT. In TNF-Rp55^{-/-}, the gene expression of MMP-2, -9, and -11 was significantly reduced at 6 days after injury, compared with WT, and that of MMP-13 at 1, 3 and 6 days. The tissue remodeling of skin wounds is determined by ECM production and matrix destruction by proteases; Reduced leukocyte infiltration may lead to the decrease of tissue destructive mechanisms, since leukocytes such as neutrophils and macrophages produce MMPs. Thus, in TNF-Rp55^{-/-}, the gene expression of MMPs was significantly reduced, showing that ECM degradation was less evident in TNF-Rp55^{-/-}, compared with WT, and, eventually, wound healing was rather accelerated.

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IFN- α and IL-18 synergistically enhance IFN- γ production in human NK cells: differential regulation of Stat4 activation and IFN- γ gene expression by IFN- α and IL-12

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IFN- γ , a product of NK and T cells, is a key cytokine contributing innate and adaptive immunity. IFN- γ production is induced via direct cell-cell contacts with APC and IFN- γ -producing cells or by cytokines. During microbial infections macrophage-derived IFN- α , IL-12, and IL-18 enhance IFN- γ production and Th1 response. Here we show that IFN- α in combination with IL-18 very efficiently induces IFN- γ expression also in primary, nonactivated NK cells and in NK-92 cell line. Comparison of the kinetics of IFN- γ mRNA expression in NK cells, NK-92 cells, and activated T cells stimulated with IFN- α or IL-12 revealed that, although both of these cytokines directly up-regulate IFN- γ mRNA expression, its levels remain elevated much longer with IL-12 stimulation. In both human NK and T cells, Stat4 is critical in IL-12 and IFN- α signaling. Stat4 was found to bind GAS elements from the promoter and intron regions of IFN- γ gene in response to IFN- α and IL-12 stimulation, as detected by EMSA and DNA affinity purification. We show that Stat4 activation is transient in cells stimulated with IFN- α , whereas IL-12 induces more long-lasting activation of Stat4. Our results demonstrate that IFN- α is an important innate cytokine, in addition to IL-12, in inducing NK cell IFN- γ production.

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Direct Cytokine mRNA Quantitation with the Invader[®] Assay

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We are presenting a method for direct quantitation of cytokine mRNA in induced U937 cells. The detection format, termed the mRNA Invader Assay, is

based on the ability of a 5' nuclease (Cleavase[®] enzyme) to recognize a specific nucleic acid structure composed of two oligonucleotides that are hybridized to the mRNA target. The signal accumulates linearly at a rate proportional to the target level present in the sample. The Invader assay is an accurate and easy method for directly and quantitatively detecting mRNA in a total RNA or cell lysate samples without further target amplification. We describe a biplex version of the Invader assay that utilizes FRET detection to quantitatively measure two distinct mRNAs in a single reaction. This format enables simultaneous expression analysis of reference housekeeping and a cytokine gene to accurately monitor the kinetics of gene expression. In the present study, the human monoblast cell line U937 induced with PMA or PMA+LPS is used as a model to measure quantitatively the mRNA levels of several cytokines (e.g., hTNF- α , hIL-1 β , hIL-6) and cFOS and cJUN transcription factors during a time course of induction. The target signal is normalized with that of T-Cell Cyclophilin or ubiquitin (internal standard) to account for sample and assay errors and to permit inter-assay comparisons. In addition, we evaluated the effect of dexamethasone on PMA induction of cytokine mRNA expression levels.

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Inducer-specific enhanceosome formation controls TNF-alpha gene expression in T lymphocytes

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We demonstrate that inducer-specific regulation of human TNF-alpha gene transcription in T cells by virus infection or intracellular calcium influx involves the recruitment of distinct enhancer complexes that include NFAT, Sp1, ATF-2, c-jun, or Ets/Elk to a shared set of transcription factor binding sites. Furthermore, depending upon the particular stimulus, distinct helical phasing relationships between transcription-factor binding sites on the TNF-alpha promoter are required for gene activation. Thus, inducer-specific TNF-alpha gene regulation in T cells involves the assembly of distinct higher order transcription enhancer complexes (enhanceosomes) depending upon the stimulus. These studies demonstrate that through the recruitment of unique enhanceosomes to shared promoter elements, a high level of transcriptional specificity is achieved in T cells in response to distinct extracellular signals.

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MCMV INFECTION DOWN-REGULATES CYTOKINE GENE EXPRESSION IN MICROVASCULAR ENDOTHELIAL CELLS

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CMV infects vascular endothelium and CMV infections have been linked to the development of several vascular diseases in humans. Infection of mice with MCMV also leads to the development of vascular disease that is characterized by the formation of neointima. We utilized microvascular endothelial cell line PY-4-1 to investigate molecular events, triggered by MCMV infection of this cell type, that may play a role in the development of vascular disease. PY-4-1 cells infected with MCMV expressed higher levels of adhesion molecules ICAM-1, E-Selectin and P-Selectin as assessed by FACS analysis. The gene expression of proinflammatory cytokines was investigated by RPA. These results indicated constitutive expression of the cytokines IL-6 and TGF- β 1, as well as chemokines RANTES, MIP-2, IP-10 and MCP-1 by control PY-4-1 cells. MCMV infection of these cells resulted in the down-regulation of mRNA levels for these cytokines as early as 1 day after infection, and this suppression was sustained during a time course of 9 days. This downregulation of cytokine gene expression is a cell type specific phenomenon since MCMV infection of 3-T12 fibroblast cells induced the expression of chemokines RANTES and MCP-1. At the same time, infection of PY-4-1 cells with MCMV resulted in the induction of TGF- β 3 mRNA. These results indicate that suppression of cytokine gene expression in the host cell may be another strategy utilized by CMV to evade immune detection. Understanding these cell type specific events may lead to better understanding of vascular diseases induced by viral infections.

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hBD-2 gene regulation in epithelial cells by macrophage-derived pro-inflammatory cytokines

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The innate immune system is the first line of host defense against invading microorganisms. In the lung, epithelial cells and macrophages respond to microbial infection, and produce active effectors including antimicrobial peptides and pro-inflammatory cytokines. Among these effectors, human β -defensin-2 (hBD-2), a cationic microbicidal peptide, is assumed to be a linking molecule between innate and acquired immunities, and its expression is induced by pro-inflammatory cytokines. Thus, we investigated the mechanisms of hBD-2 gene regulation in epithelial cells by pro-inflammatory cytokines. Luciferase assay revealed that the hBD-2 promoter activity in A549 lung epithelial cells was significantly increased in response to TNF- α , but not IL-6 or IFN- γ . In addition, deletion and mutation analyses of hBD-2 promoter indicated that tandemly repeated NF κ B motifs at -200 were crucial for TNF- α responsiveness in A549 cells. Furthermore, gel shift analysis with TNF- α stimulated A549 nuclear extracts showed that NF κ B p65-p50 heterodimer could bind to the NF κ B motifs. Interestingly, co-culture of A549 cells with monocytic Mono-Mac-6 cells in presence of *E. coli* LPS remarkably induced the hBD-2 promoter activity, although A549 cells alone did not respond to LPS. Apparently, Mono-Mac-6 cells expressed LPS receptors (CD14 and TLR4) and produced TNF- α following LPS stimulation, while A549 cells did not. These results suggest that the hBD-2 expression in epithelial cells is likely mediated by macrophage-derived pro-inflammatory cytokines (such as TNF- α).

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Identification of transcriptional domains of human lactoferrin and its roles in transcription of human IL-1 β gene in mammalian cells

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Lactoferrin (Lf), an iron-binding glycoprotein predominantly found in milk is also present in other human body secretions and in the granules of polymorphonuclear leukocytes. Lf is a multifunctional protein which plays roles in primary defense against microbial infection and also in many other cellular responses such as immunomodulation and cell growth regulation. Some of these functions are independent of iron binding property of Lf and supposed to be related to binding of Lf to specific DNA. Lf is known to bind to DNA, and specific DNA sequences to which Lf binds have been reported previously. Lf is able to activate transcription of a reporter gene under the control of Adenovirus E1b minimal promoter containing one of Lf binding site, GGCACCTTGC. In the present study, we have identified functional domains of human Lf which directs transcription of a reporter gene containing four tandem repeat sequences of Lf binding sites (LBS). The N-terminal 90 amino acids, called N1a domain and the N-terminal half lobe between amino acid residue 1 and 345 were able to act as a transcriptional activator equally well as the intact Lf, while the C-terminal half lobe between residues 342 and 692 had little activity. The C-terminal half lobe binds to the LBS but lacks the transactivating activity. Gel mobility shift assay demonstrated that the N-terminal 90 amino acid region was bound to the Lf binding site. We also showed that the 90 amino acid region contains transcription activation domain by using GAL4 DNA binding domain fusion plasmids in mammalian cells. In order to confirm that Lf plays roles in transcription of any cellular genes in the cells, we searched for genes containing LBS by database analysis and found that human IL-1 β gene among a number of genes contains five putative LBS in the 5'-flanking sequence. Treatment of K562 cells with phorbol myristate acetate (PMA) and Lf resulted in the higher level of IL-1 β mRNA than that of the cells treated with PMA alone. The results were confirmed by similar treatments of the transfected cells expressing human Lf and IL-1 β /Luciferase reporter genes. Our results indicate that transcription of IL-1 β gene may be activated by Lf in conjunction with other transcription factors.

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TGF β selectively regulates LPS-induced chemokine gene transcription
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TGF β is widely recognized as an anti-inflammatory agent that can selectively diminish pro-inflammatory gene expression in multiple cell types. Amongst the genes exhibiting sensitivity to TGF β are members of the chemoattractant cytokine gene family responsible for recruiting distinct populations of leukocytes to sites of inflammation. The mechanisms involved in mediating the anti-inflammatory action of TGF β are diverse and poorly understood at the present time. TGF β can inhibit LPS-stimulated expression of the CXC chemokines KC and MIP-2 in mouse macrophages by 60-80% but has no effect on expression of MCP-1 or IP-10. These levels of KC and MIP-2 mRNAs are minimally altered at 1 hr after addition of LPS and TGF β but are markedly reduced following 3-4 hrs of stimulation. While the rates of KC and MIP-2 mRNA degradation are comparable in both untreated and TGF β treated cultures, the rates of LPS-stimulated KC and MIP-2 transcription were markedly reduced by TGF β but only after 2 hrs of stimulation. Hence TGF β appears to block chemokine gene transcription well after the initiating signal. Analysis of the KC genomic sequence suggests that regulatory sequence within transcribed exons confers transcriptional sensitivity to TGF β . Although a functional promoter is necessary, the KC promoter is not specifically required. This work supported by USPHS grant CA62220

POSTTRANSLATIONAL REGULATION (142-143)

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UNSTIMULATED HUMAN CD4 LYMPHOCYTES EXPRESS A CYTOPLASMIC IMMATURE FORM OF THE COMMON CYTOKINE RECEPTOR γ CHAIN

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As a component of various cytokine receptors, common cytokine receptor γ chain (γ c) is essential in the development of the immune system and plays an important role in different stages of inflammatory and immune responses. Here we establish that resting CD4 T cells and the Jurkat CD4 T cell line do not express the mature form of γ c (64 kDa) recognized by monoclonal antibody Tugh4. However, these cells constitutively transcribe the corresponding γ c gene. This apparent paradox was solved by the demonstration that polyclonal anti- γ c antibodies detected a Endo-H sensitive immature forms of γ c (54-58 kDa) expressed by quiescent CD4 T lymphocytes and Jurkat cells. Immature γ c is characterized as an intracellular component localized in the endoplasmic reticulum. Pulse-chase analysis shows that the immature γ c is rapidly degraded after synthesis. After activation of CD4 T lymphocytes, and as seen in the CD4 T cell line Kit 225, the Endo-H resistant mature form of γ c is detectable at the cell surface and in the endosomal compartment. For the first time, our results demonstrate that a cytokine receptor chain may be constitutively produced as an immature form. Furthermore, this supports the notion that expression of the functional form of γ c may require intracellular interactions with lineage or subset-specific molecular partners.

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Stimulus-coupled IL-1 β posttranslational processing: Identification of a novel class of inhibitors.

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IL-1 β is synthesized by monocytes/macrophages as an inactive, signal peptide-deficient, 31 kDa polypeptide in response to inflammatory stimuli such as LPS. For IL-1 β to bind to its receptor on target cells, the propolypeptide must

be cleaved to a mature 17 kDa species and externalized. A cysteine protease found in the cytosol, caspase-1, converts proIL-1 β to its active form. Caspase-1, although constitutively present, also is synthesized as an inactive precursor and must be activated in order to process proIL-1 β . Mechanisms that control activation of caspase-1, cleavage of proIL-1 β , and release of 17 kDa IL-1 β are not well understood. Recent studies, however, indicate that these posttranslational processing reactions require the cytokine producing cells to encounter a "secretion stimulus". For example, LPS activated monocytes release little IL-1 β to the medium, but the subsequent addition of extracellular ATP to the monocyte cultures causes rapid formation and release of the 17 kDa species. Two novel diarylsulfonylurea-containing compounds act as selective cytokine release inhibitory drugs (CRIDs) that block ATP-induced posttranslational processing and release of 17 kDa IL-1 β without increasing release of proIL-1 β . In contrast, caspase-1 inhibitors block formation of 17 kDa IL-1 β , but the ATP-activated cells may continue to release pro-IL-1 β and mature caspase-1 to the medium. CRIDs are specific in their action as secretion of TNF α , IL-6, and IL-1 receptor antagonist are not affected. Thus, CRIDs represent a novel therapeutic approach for regulating IL-1 and, in turn, for controlling an inflammatory process.

APOPTOSIS (144-153)

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TIAF1 is an effector of transforming growth factor- β -mediated growth suppression and promotion

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The mechanisms by which TGF- β mediates growth suppression and apoptosis of epithelial and liver cells but supports fibroblast proliferation are unknown. TGF- β -induced antiapoptotic factor (TIAF1) protects murine L929 fibroblasts from TNF cytotoxicity. However, like TGF- β , TIAF1 mediates growth inhibition, differentiation and apoptosis of monocytic U937 cells due in part to TIAF1-induced increases in p53 and p21/Cip1 expression and reduction in ERK phosphorylation. Also, like TGF- β , overexpression of TIAF1 does not induce apoptosis of fibroblasts but does support transforming growth of these cells. Antisense TIAF1 mRNA dramatically enhances the proliferation of TGF- β -sensitive mink lung epithelial Mv1Lu cells. These functional similarities with TGF- β indicate that TIAF1 is a downstream effector of the growth inhibiting and promoting effects of TGF- β . TIAF1 is present in mitochondria and physically interacts with mitochondrial WW domain-containing oxidoreductase (WOX1). WOX1 is known to bind p53 and is an essential partner of p53 in apoptosis. Fibroblasts can be sensitized to TGF- β -mediated apoptosis by TIAF1 in combination with p53 or WOX1. When Cos7 fibroblasts are cotransfected with p53, WOX1 and TIAF1, the expressed proteins colocalize in the mitochondria, migrate to the nuclei in response to TGF- β , and mediate cell death at the nuclear level. These observations suggest that WOX1 connects the binding interactions between p53 and TIAF1. TGF- β -mediated cell death is involved in mitochondrial release and nuclear translocation of the p53/WOX1/TIAF1 complex.

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The Non-Ankyrin C-Terminus of I κ B α Physically Interacts with p53 In Vivo and Inhibits Transforming Growth Factor- β -Mediated Growth Suppression

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Transforming growth factor beta (TGF- β) suppresses the growth of mink lung Mv1Lu epithelial cells, whereas testicular hyaluronidase abolishes the growth inhibition. Exposure of Mv1Lu cells to TGF- β rapidly results in downregulation of I κ B α expression and hyaluronidase prevents the effect, suggesting that I κ B α may play a role in the TGF- β -mediated growth suppression. Ectopic expression of both wild type and dominant negative

I κ B α enables Mv1Lu cells to resist growth suppression by TGF- β . Nonetheless, the I κ B α effect is not related with regulation of NF- κ B function by its N-terminal ankyrin-repeat-containing region (amino acid #1-243). Removal of the PEST domain-containing C-terminus (amino acid 244-314) abolishes the I κ B α function and the C-terminus alone blocks the TGF- β growth-inhibitory effect. Immunoprecipitation revealed that endogenous I κ B α physically interacts with p53 in the cytosol but not in the nucleus. Yeast two-hybrid analysis showed that the non-ankyrin C-terminus of I κ B α binds the proline-rich (or growth regulatory) region of p53. Since p53 is involved in TGF- β -mediated growth inhibition and apoptosis, the binding of p53 by I κ B α in vivo is likely to regulate p53-mediated growth arrest and apoptosis.

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Modulation of leukotriene B4 receptor-1 expression by dexamethasone: potential mechanism for enhanced neutrophil survival

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Glucocorticoids can downregulate many inflammatory and immune responses and constitute a powerful therapeutic tool in a number of diseases. They have, however, a somewhat paradoxical effect on neutrophils, in that they prolong their survival. Since leukotriene (LT)B4 can also extend neutrophil survival, we proposed that glucocorticoids could prevent neutrophil apoptosis by upregulating their expression of the high affinity leukotriene B4 receptor (BLT1). Here we show that, indeed, dexamethasone (DEX) upregulates the steady-state levels of BLT1 mRNA in human neutrophils. The effect was time and concentration-dependent, being maximal at 8 h and at 10- 100 nM DEX. The effect was also dependent on transcriptional activity, whereas BLT1 mRNA stability was not affected. BLT1 protein expression on neutrophils exposed to DEX for 24 h was also upregulated 2- to 3-fold, and DEX-treated cells showed enhanced responsiveness to LTB4 in terms of intracellular Ca⁺⁺ mobilization and chemotaxis. Whereas both DEX and LTB4 induced neutrophil survival by approximately 50%, neutrophils treated with both LTB4 and DEX showed greater than 90% survival at 24h. Taken together, our results suggest that DEX-induced upregulation of BLT1 expression in neutrophils may be one mechanism through which glucocorticoids can prolong neutrophil survival, namely by enhancing cell responses to the anti-apoptotic effect of LTB4.

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Potential by Human Serum of Anti-inflammatory Cytokine Production by Human Macrophages In Response to Apoptotic Cells

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Phagocytosis of apoptotic cells by macrophages leads to the production of anti-inflammatory cytokines, thereby preventing inflammation. In this study we demonstrate that human serum potentiates the production of anti-inflammatory cytokines, IL-10 and TGF- β , by PMA-treated THP-1 cells and human monocyte-derived macrophages in response to apoptotic cells, which results in great suppression of the production of pro-inflammatory cytokine, IL-8. Human IgG but not its F(ab)₂ suppressed the IL-8 production. Pretreatment of macrophages but not apoptotic cells with either human serum or human IgG caused the suppression, suggesting that immune complex may not be formed with apoptotic cells. When Fc γ RI was specifically down-modulated by a monoclonal antibody, M22, both the potentiating effects of human serum and human IgG on the anti-inflammatory cytokine production and the suppressive effects on IL-8 production were completely abolished. Thus human IgG and Fc γ RI appear to be critical in leading to production of anti-inflammatory cytokines by macrophage in response to apoptotic cells.

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TGF- β 1 IS A TARGET MOLECULE IN BISPHOSPHONATE-INDUCED APOPTOSIS IN HUMAN NEOPLASTIC CELLS

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Bisphosphonates (BP) are potent antiresorptive compounds with a beneficial effect in reducing skeletal metastasis in patient with cancer. However, there is no clear information on the direct effect of BP on tumor cells. On the other hand, transforming growth factor-beta (TGF- β) is present at high concentrations in bone matrix and plays a regulatory function in tumor growth and metastasis. Therefore, we investigated the direct effect of BP on human tumor cells and on TGF- β expression. Human osteosarcoma cells (HOS) were treated with BP-Alendronate (ALN) or with TGF- β 1. Proliferation assays showed an inhibitory effect of ALN, while TGF- β had an enhancing effect on cell proliferation. ALN-treated cells retracted, became round, lost adhesion to the surface and fragmented. Hoechst fluorochrome, Annexin V and propidium iodide staining showed the typical features of apoptotic cell death. DNA extraction and gel electrophoresis revealed DNA fragmentation and laddering. Cells were also treated before or after adhesion to the culture plates, and in medium supplemented with low or high calcium concentrations. Results confirmed that the effect of ALN was not due to an interference with cell adhesion or due to calcium chelation. RT-PCR analysis and immunoassays showed that the growth inhibitory effect of ALN on the tumor cells is associated with downregulation of TGF- β 1 mRNA and inhibition of TGF- β 1 protein production. In conclusion, Bisphosphonate (ALN) inhibits proliferation and induces apoptosis in human osteosarcoma cells through a mechanism which involves downregulation of TGF- β 1 expression.

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Sayrin-1 is an antiapoptotic factor that blocks TNF and Fas-mediated cell death

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By functional cloning, here we report the isolation of a murine 7-transmembrane domain antiapoptotic protein, designated sayrin-1. The murine sayrin-1 protein sequence shares 74.7% identity with that of the human protein TM7SF3 (transmembrane 7 superfamily member 3). However, unlike the cell-membrane localized human TM7SF3, the murine sayrin-1 localizes in the cytosol, as determined by immunostaining and expressing the protein with a GFP-tag. Sayrin-1 blocks cell death by TNF and anti-fas antibody. Sayrin-1 migrates to the nuclei in response to stimulation by death stimuli, suggesting that sayrin-1 blocks cell death at the nuclear level. Yeast two-hybrid analysis is being underway to isolate the sayrin-1 binding protein.

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TNF sensitizes Fas-mediated apoptosis irrespective of its anti-apoptotic effects in follicular dendritic cells

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It is well recognized that TNF not only initiates signaling pathway leading to apoptosis but also to cell survival, through activation of NF- κ B. We investigated the role of TNF in a follicular dendritic cell (FDC) line, HK cells. We found that TNF is a mitogenic growth factor for HK cells and shows a prolonged NF- κ B activation even after 24 hours of treatment. As a result, anti-apoptotic genes including TRAF1, TRAF2, c-IAP1 and c-IAP2 were persistently induced in HK cells. Although TNF treatment led to the cleavage of caspase-8 and -9, their activities appeared to be blocked by the concerted action of the induced anti-apoptotic proteins as evidenced from the observation that HK cells were resistant to the combination treatment of TNF plus cycloheximide. However, these TNF-induced anti-apoptotic proteins could not

protect HK cells from cell death induced by UV-irradiation and Fas stimulation. Rather, TNF pretreatment accelerated these cell death through the up-regulation of Fas. In addition, the cleaved caspase-8 and -9 in TNF-treated cells sensitized Fas-mediated cell death. Overall, our observation suggests that TNF is involved in the formation of FDC network and its resolution during the germinal center reaction.

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A Molecular Basis for the Endotoxin Resistance of MIF-knockout Mice: MIF Inhibits Cellular p53 Accumulation via Cox-2 Induction.

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Macrophage migration inhibitory factor (MIF) exists preformed in monocytes/macrophages and exerts critical autocrine/paracrine activating effects on a variety of cell types. MIF is critically involved in the pathophysiology of septic shock and other inflammatory conditions, a finding that has been affirmed by the endotoxin resistant phenotype of genetically-deficient (MIF-KO) mice. MIF also has been reported recently to inhibit p53-dependent apoptosis in vitro. We have observed that endotoxin administration to MIF-/- mice results in decreased macrophage viability, decreased pro-inflammatory function, and increased apoptosis when compared to wild-type controls. Inhibition of macrophage p53 by MIF coincides with the induction of arachidonic acid metabolism and Cox-2 expression, which is required for MIF regulation of p53 accumulation in cells. The role of Cox-2 activity in MIF's modulation of p53 was verified by replacing the requirement for MIF's inhibition of macrophage apoptosis by prostaglandin E2 in MIF knockout cells. Moreover, TAT-transducible dominant-negative p53 treatment of MIF-deficient macrophages suppressed enhanced activation-induced apoptosis and fully restored pro-inflammatory function. MIF's affect on macrophage viability and survival provides a mechanism to explain the endotoxin resistance of MIF-deficient mice, and underscores the potential importance of apoptotic mechanisms in the pathophysiology of septic shock.

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REGULATION OF APOPTOTIC CELL DEATH BY INTERLEUKIN-6 AND ONCOSTATIN M

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Interleukin-6 (IL-6) was described as an autocrine growth factor for various cell types. STAT3, a major signal transducer of IL-6, has also been implicated in the prevention of apoptosis. STAT3 is involved in the suppression of apoptosis in a pro-B cell line, myeloma cells and also in T cells. In contrast to this, we show that IL-6 enhances susceptibility to TNF-mediated cell death in epithelial cell lines. Sensitization to TNF apoptosis was not only induced after IL-6 stimulation but also after application of the gp130-activating cytokine oncostatin M. Both cytokines strongly activated STAT3 in these cells. As judged by RNase protection analysis, enhanced TNF-mediated apoptosis was not due to the up-regulation of the TNF receptor (TNFR) or associated factors. IL-6 and OSM did not lead to a general enhancement of TNF functions. Thus, neither nuclear factor κ B (NF- κ B) activation, nor TNF-mediated induction of the chemokine IL-8 involving a variety of transcription factors including NF- κ B, NF-IL6 and activator protein-1 (AP-1) were affected. Also, IL-6 and OSM did not change the expression of caspases involved in TNF-mediated cell death. This was substantiated by the fact that the cytokines did not sensitize for Fas-mediated cell death demonstrating that they had no effect on the apoptosis effector pathway common for Fas and the TNFR. In conclusion, IL-6 and OSM specifically sensitize epithelial cells for TNF-mediated apoptosis. The molecular mechanism of sensitization is currently investigated.

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CHARACTERIZATION OF A T-CELL RECEPTOR SIGNALING-DEFICIENT JURKAT CELL VARIANT THAT EXHIBITS TYPE I INTERFERON INDUCED APOPTOSIS

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Expression of CD45, Lck or ZAP-70 are required for the antiproliferative effects of IFN- α in Jurkat T cells. Screening of several other Jurkat cell variants that were mutagenized with ICR-191 and found to be defective in TCR stimulated increase in intracellular calcium led to the isolation of a Jurkat variant, H123. Stimulation of parental Jurkat cells with IFN- α slows the growth of these cells 30 to 50% in the absence of programmed cell death. Incubation of H123 cells with IFN- α induces 90% of the cells to apoptose within 72 hrs. IFN- α activation of the Jak/Stat pathway is the same in both cell lines. To identify candidate genes that might regulate the apoptotic actions of IFN- α in H123 cells, we performed a gene chip differential display using mRNAs from untreated and IFN- α treated parental and H123 Jurkat cells. Data analysis revealed several genes that were not expressed in H123 cells (i.e. S10/Rab33A and MAL). Interestingly, the basal mRNA expression of granzyme A, which plays a role in apoptosis, was higher in H123 cells compared to parental Jurkat cells. The level of expression of this gene was enhanced upon IFN- α treatment in both cell lines. We are currently in the process of reconstituting H123 Jurkat cells with the missing genes to determine whether expression of any of these genes revert these cells to the wild type phenotype and protect them from IFN- α induced apoptosis. These results will provide evidence on what genes are required for type I IFNs to decide between the induction of apoptosis and antiproliferative responses.

CYTOKINE AND CHEMOTACTIN RECEPTORS (154-156)

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Serum soluble macrophage colony-stimulating factor receptor level in patients with disease of cardiovascular system and kidneys

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Macrophage colony-stimulating factor (M-CSF), initially described as a growth factor of the mononuclear phagocytic lineage, also participates in immunological and inflammatory reactions. Increasing evidences suggest that M-CSF may play a role in chronic inflammatory and autoimmune disease. The serum levels of soluble M-CSFR (M-CSFRs) in the patients with digestive system diseases, kidney diseases and cardiovascular system diseases were investigated via M-CSFR specific sandwich ELISA developed in our lab. Serum levels of M-CSFRs (mean \pm SD, ng/ml) were significantly higher in 55 cardiovascular disease patients (1.19 \pm 1.38, $p=0.003$) and significantly lower in 78 patients with kidney diseases (0.17 \pm 0.49, $p < 0.001$) than in 134 healthy volunteers (0.59 \pm 0.78). No significant difference was found between M-CSFRs level in digestive system disease patients and in healthy volunteers. For cardiovascular disease patients, 28 arteriosclerosis patients showed significantly higher serum M-CSFRs levels (1.68 \pm 1.45, $p=0.001$). Patients with other types of cardiovascular disease showed normal serum level. Otherwise, saliva M-CSFRs levels in seven coronary heart disease patients were examined. M-CSFRs was detected in saliva of three patients and the mean saliva M-CSFRs level was 0.63ng/ml. For 78 kidney diseases patients, 37 chronic renal failure patients had the lowest serum M-CSFRs levels (0.08 \pm 0.18, $p < 0.001$) and 21 chronic glomerulonephritis patients had lower serum M-CSFRs levels (0.22 \pm 0.34, $p=0.01$). Compared with normal level, other types of kidney diseases showed lower serum M-CSFRs levels but no significant difference was found. Soluble cytokine receptors (sCR) contribute to regulation of cytokine activities in vivo. Evidences show that level of certain sCR in serum correlate with immunologic activation or disease. Our data suggests that serum M-CSFRs level correlate with arteriosclerosis, chronic renal failure and chronic glomerulonephritis. Excessive levels and low-levels of M-CSFRs could contribute or even result from arteriosclerosis and renal diseases.

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Inflammatory Stimuli Regulate the Secretion of the Soluble Human GM-CSF Receptor by Monocytes and Granulocytes

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A soluble GM-CSF receptor protein (sGMRa) was recently identified in human plasma and in media conditioned by human granulocytes and myeloid leukemic cell lines. In this abstract we demonstrate that monocytes, but not lymphocytes or vascular endothelial cells, can also secrete sGMRa. Importantly, we demonstrate for the first time that inflammatory stimuli can moderate the secretion of sGMRa by both granulocytes and monocytes. Monocytes secreted sGMRa in vitro for up to 96 hours, while unstimulated granulocytes did not, suggesting that monocytes constitutively secrete sGMRa and granulocytes do not. Stimulation of monocytes with a variety of inflammatory mediators such as PMA, A23187, LPS and GM-CSF resulted in a rapid but transient increase in sGMRa secretion, followed by inhibition of long-term constitutive sGMRa secretion. Stimulation of granulocytes with the same inflammatory stimuli also induced a rapid yet transient increase in sGMRa secretion. These observations suggest that sGMRa secretion by monocytes and granulocytes is specifically regulated by inflammatory stimuli and suggests that sGMRa may be involved in moderating GM-CSF function during inflammation.

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The role of galectin-3 in macrophage spreading.

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Galectin-3 (Gal3) is a β -galactoside-binding protein with monocyte chemotactic and chemokinetic activity. We have investigated the role of this protein on macrophage spreading on different surfaces. Thioglycollate-induced peritoneal macrophages from Gal3 knockout (Gal3KO) and 129/J strain mice were grown in RPMI-1640 medium supplemented with fetal bovine serum on tissue culture plastic (TCP), fibronectin (FN), or heat-denatured collagen (COL) surface and their spreading was measured at different times over 72 hours. Spreading of Gal3KO mouse macrophages was significantly lower than that of 129/J macrophages at 24 hours, regardless of the surface on which they were cultured. While spreading of both types of macrophages was lower on COL than on TCP or FN, only spreading of Gal3KO macrophages was significantly lower on this surface, even at 72 hours. Spreading of Gal3KO macrophages on TCP increased significantly after treatment with recombinant Gal3 (50 mg/ml). Treatment of these macrophages with fetuin or asialofetuin did not change their spreading. Furthermore, this effect of Gal3 on Gal3KO macrophage spreading was maintained in the presence of lactose (25 mM), a natural ligand for the carbohydrate-binding domain of Gal3. These data suggest that Gal3 may play a role in the spreading of macrophages to solid surfaces (extracellular matrix), probably by causing changes in the cytoskeleton.

TRANSGENIC/KNOCKOUT (157-158)

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The Rescue of SSI-1/SOCS-1 KO Mice by Lymphoid-specific Restoration of SSI-1/SOCS-1 Expression

M. Fujimoto, T. Naka, H. Tsutsui, E. Seki, M. Himeno, T. Abe, A. Kimura, K. Nakanishi & T. Kishimoto. Dept. of Molecular Medicine, Osaka University, Osaka, JAPAN & Dept. of Immunology and Medical Zoology, Hyogo, JAPAN. SSI-1 (STAT-induced STAT inhibitor-1)/SOCS-1 (Suppressor of cytokine signaling-1) is an inhibitor of cytokine signaling that appears to function in a negative feedback manner. SSI-1 KO mice die before 3 weeks of age from complex multi-organ disease including lymphoid atrophy and fulminant

hepatitis. Predominant expression of SSI-1 in lymphoid organs and the rescue of SSI-1 KO mice from lethality by simultaneous disruption of Rag2 gene suggest a critical role of lymphocytes in the pathology of SSI-1 KO mice. Therefore, we attempted to rescue SSI-1 KO mice by introducing two types of SSI-1 transgenes (Lck-SSI-1 and E μ -SSI-1) that restore the expression of SSI-1 specifically in the lymphoid lineage. Interestingly, most of E μ -SSI-1/SSI-1 KO mice survived longer than SSI-1 KO mice, confirming the vital role of SSI-1 in lymphocytes but less in other cell types. However, Lck-SSI-1/SSI-1 KO mice showed perinatal lethality similar to that of SSI-1 KO mice. This difference appears to be attributable to the characteristics of the two promoters (Lck and E μ) and further analyses are currently underway to reveal the significance of SSI-1 expression in lymphocytes.

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Self-deactivating macrophages: transgenic overexpression of IL-10 directed by the CD68 promoter impairs inflammatory macrophage responses

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The type and intensity of macrophage responses to infectious and inflammatory stimuli is regulated by a complex set of genetic and environmental factors. Interleukin-10 (IL-10) is a major downregulator of cytokine production by macrophages. We asked what consequences constitutive overexpression of IL-10 from macrophages would have on innate and adaptive immune responses by generating transgenic mice (macIL-10tg mice) expressing Flag-tagged IL-10 (Flag-IL-10) under control of the CD68 promoter. macIL-10tg mice were fertile and appeared healthy. FACS-sorted macrophages from spleen produced high levels of Flag-IL-10, but T and B lymphocytes did not, demonstrating the macrophage-specificity of the CD68 promoter. *In vitro* stimulation of transgenic macrophages resulted in strongly suppressed production of TNF α and IL-12, which was partially restored by a neutralizing anti-IL-10. When the mice were challenged with LPS *in vivo*, serum levels of TNF α , IL-12p40 and IFN γ were significantly lower in macIL-10tg mice than in controls. Anti-mycobacterial immunity was analyzed in macIL-10tg mice after infection with *M. bovis* BCG. While macIL-10tg mice controlled infection, bacterial loads in organs were approx. 10-fold higher than in control mice, indicating impaired killing of mycobacteria. Taken together, macIL-10tg mice have deactivated macrophages and should prove to be a valuable tool in studying the role of macrophage activation status not only in the immunology of infection but also other fields such as atherosclerosis and tumor immunology.

RECEPTOR-LIGAND INTERACTIONS (159-160)

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Cognate interactions mediated by a recombinant LFA-3/IgG1 fusion induce CD16 signaling and granzyme B dependent apoptosis of activated CD2+ cells.

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Alefacept, a bivalent recombinant fusion protein composed of the first extracellular domain of lymphocyte function-associated antigen 3 (LFA-3) fused to the hinge, CH2 domain, and CH3 domain of human IgG1. This LFA-3/IgG1 fusion protein modulates the function of and induces apoptosis of human CD2+ cells *in vitro* and *in vivo*. Experimentation using mutant LFA-3/IgG1 isoforms and cell depletion or antibody blockade strategies indicate that alefacept immunomodulation reflects its ability to mediate cognate interactions between cells expressing human CD2 and human CD16 (Fc γ RIII). To address the molecular and structural basis for the mechanisms of action of alefacept we have investigated its signaling inducing properties in both transfected Jurkat cells as well as in IL-2 expanded NK cells which express both CD2 and CD16. Alefacept induces the activation of intracellular signaling pathways, e.g. increases p42/44MAPK (ERK) phosphorylation, up-

regulates expression of cell surface activation marker CD25, and induces release of Granzyme B. Importantly, the data suggest that, although binding to CD2 is required, most if not all of the signaling is induced through CD16. Thus alefacept acts as an effector molecule, mediating cognate interactions of cells expressing CD2 and Fc γ R to activate Fc γ R+ cells (e.g. CD16+ NK cells). These cells secrete granzyme/perforin to induce activated CD2+ target cells (e.g. T-cells) to undergo apoptosis as measured by Annexin V staining.

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IL-18 mutants with enhanced biological activities and decreased IL-18BP neutralization

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Interleukin-18 (IL-18) can be considered a pro-inflammatory cytokine mediating disease as well as an immunostimulatory cytokine important for host defense against infection and cancer. The high affinity, constitutively expressed and circulating IL-18 binding protein (IL-18BP), which competes with cell surface receptors for IL-18 and neutralizes IL-18 activity, may act as a natural anti-inflammatory as well as immunosuppressive molecule. Single point mutations in the mature IL-18 were made and the biological activities of the wild-type IL-18 were compared to those of the mutants. Mutants E42A and K89A exhibited two-fold increased activity compared to wild-type IL-18. A double mutant, E42A plus K89A, exhibited four-fold greater activity. Unexpectedly, IL-18BP failed to neutralize the double mutant E42A plus K89A compared to wild-type IL-18. The K89A mutant was intermediate in being neutralized by IL-18BP whereas neutralization of the E42A mutant was comparable to that in the wild-type IL-18. We further examined an affinity between FcIL-18BP and each of four IL-18s (WT, E42A, K89A, and E42A+K89A) with use of BIAcore. All three mutants dramatically decreased affinity from 50 to 100-fold compared to WT. The identification of E42 and K89 in the mature IL-18 is consistent with previous modeling studies of IL-18 binding to IL-18BP and these mutants could be used as a potential molecule for anti-

SIGNAL TRANSDUCTION PATHWAYS (161-174)

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Functional Dissection of the Interleukin-1 Receptor Associated Kinase domains

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Interleukin-1 receptor associated kinase (IRAK) plays a pivotal role in mediating interleukin-1 receptor /Toll-like receptor (IL-1R/TLR) signaling and innate immunity activation. IRAK from leukocytes undergoes rapid activation and degradation following interleukin-1 (IL-1) or lipopolysaccharide (LPS) stimulation. The rapid degradation of IRAK serves as a negative feedback mechanism of down-regulating IL-1R/TLR mediated signaling and cytokine gene transcription. IRAK composes of an N-terminus death domain, a central kinase domain as well as a C-terminus serine/threonine rich region. In this report, we observe that the IRAK N-terminus harbors the degradation signal. The N-terminally truncated IRAK protein expressed in human monocytic THP-1 cells remains stable upon LPS challenge. In comparison, IRAK, kinase-dead IRAK (K239A) mutant as well as the IRAK mutant with C-terminal truncation undergo degradation with LPS stimulation. Furthermore, we demonstrate that functional TLR4 receptor is required for LPS-mediated IRAK degradation. IRAK protein in the murine GG2EE cells harboring a mutated TLR4 gene does not undergo degradation upon LPS treatment. In addition, we also examined the role of various IRAK domains in NF κ B activation. We demonstrate that the N-terminus, kinase domain, as well as the C-terminus are all required for proper NF κ B activation.

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Detection of Protein Tyrosine Kinase in Immature Chicken Heterophils.

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Protein tyrosine phosphorylation is a central mechanism that mediates signal transduction events involved in many cellular processes. Protein tyrosine kinase (PTK) is the mediating enzyme in this signal transduction pathway. Previous studies from our laboratory have demonstrated a dramatic inhibition of oxidative burst and degranulation in chicken heterophils following treatment with the PTK inhibitor, genistein. These results are indicative of the critical nature of protein tyrosine phosphorylation as a signaling event during functional activity. The purpose of these experiments was to detect and quantitate PTK in heterophils isolated from day of hatch chicks with a commercial kit. Our data demonstrate a significant increase ($P < 0.05$) in the quantity of PTK present in control heterophils vs LPS stimulated heterophils isolated from day-old chicks. Quantitatively, PTK levels were significantly decreased ($P < 0.05$) between genistein treated heterophils (PTK inhibitor) and LPS stimulated heterophils. Our data demonstrate PTK is present in the heterophils of day-old chicks. The presence of PTK and our ability to detect increased and decreased levels in day-old chicken heterophils is indicative of protein tyrosine phosphorylation which mediates signal transduction events at the cellular level.

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DESIGN AND APPLICATION OF A CYTOKINE RECEPTOR-BASED INTERACTION TRAP

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Protein interactions underlie the structural and functional integrity of many subcellular processes. The notion that most of the proteins within a cell are part of higher order complexes regulating signal transduction, gene expression, apoptosis and other crucial events, becomes generally accepted. Existing methodologies for large-scale protein interaction mapping include yeast two-hybrid, phage display and mass spectrometric analysis. Although these approaches have found wide application, each of them suffers from intrinsic limitations, not at least from the fact that interactions are analyzed in a non-physiological context. We have developed a cytokine receptor-based two-hybrid method in mammalian cells. Incorporation of an interaction trap in a signalling-deficient receptor allows identification of protein-protein interactions using a STAT-dependent complementation assay. Interaction between 'bait' and 'prey' leads to ligand-dependent STAT activation, which is detected by the use of STAT-dependent reporter/selector genes. Using this mammalian protein-protein interaction trap (MAPPIT) we were able to demonstrate both modification independent and tyrosine-phosphorylation dependent interactions. Data will be presented on the application of this concept as a screening tool. This MAPPIT procedure places protein-protein interactions in their normal physiological context and may be particularly instrumental for the *in situ* analysis of signal transduction pathways.

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TLR2 AND TLR4 LIGANDS DIFFERENTIALLY REGULATE IL-1Ra GENE EXPRESSION IN MACROPHAGES

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Treatment of macrophages with lipopolysaccharide (LPS) from gram negative bacteria or peptidoglycan (PGN) from gram positive bacteria activates

multiple intracellular signaling pathways and a large, diverse group of nuclear transcription factors. The signaling receptors for PGN and LPS are now known to be the Toll-like receptors (TLR) 2 and 4, respectively. While a large body of literature indicates that the members of TLR family activate nearly identical cytoplasmic signaling programs, several recent reports have suggested that the functional outcomes of signaling via TLR2 or TLR4 are not equivalent. In the current studies we compared the responses of the sIL-1Ra gene to both LPS and PGN. Both LPS and PGN induced IL-1Ra gene expression however the combination of both stimuli synergistically increased sIL-1Ra mRNA expression and promoter activity, suggesting that the signals induced via TLR2 and TLR4 are not equivalent. While both LPS and PGN utilized the PU.1-binding sites in the proximal promoter region, additional distinct promoter elements were utilized by LPS or PGN. Activation of p38 SAPK was required for LPS or PGN-induced IL-1Ra gene expression but the p38-responsive promoter elements localized to distinct regions of the sIL-1Ra gene. Additionally, while the LPS-induced, p38-dependent required PU.1 binding, the PGN-induced, p38 response did not. Collectively, these data indicated that while some of the intracellular signaling events occurring downstream of TLR2 and TLR4 are similar, there are clearly distinct differences in the responses elicited by ligands specific for these two Toll-like receptors.

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Regulation of TNF α and Caspases by IFN γ results in Differential MMP-1 and MMP-9 Production by GM-CSF and TNF α Treated Monocytes

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We have previously shown that TNF α and GM-CSF individually enhance monocyte matrix metalloproteinase-9 (MMP-9/92 kD gelatinase) but induce MMP-1 (interstitial collagenase) only when added in combination. Since IFN γ is also found at inflammatory sites, we determined its effect on monocyte MMPs in the presence or absence of other cytokines. While IFN γ alone did not induce monocyte MMP-9 or MMP-1, it differentially regulated MMP-1 and MMP-9 in the presence of GM-CSF and/or TNF α . IFN γ significantly enhanced MMP-1 induced by GM-CSF and TNF α . Of particular interest was the induction of monocyte MMP-1 when IFN γ was added in combination with GM-CSF. We show that IFN γ when combined with GM-CSF, but not alone, stimulated the production of TNF α , thus resulting in the induction of MMP-1. This conclusion was further supported by the ability of TNF α neutralizing antibodies to block the induction of MMP-1 by GM-CSF and IFN γ . In contrast to its effects on MMP-1, IFN γ inhibited TNF α -induced MMP-9. This inhibitory effect was mediated through a caspase-dependent pathway as demonstrated by the restoration of MMP-9 with caspase inhibitors. These findings indicate that the influence of IFN γ on monocyte modulation of connective tissue metabolism is complex and most likely determined by the specific cytokines present in an inflammatory lesion.

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THE C-TERMINAL PART OF IL-10 IS REGULATING PROLIFERATION AND APOPTOSIS IN HEPATO-CELLULAR CARCINOMA CELLS THROUGH A P53 MECHANISM.

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IL-10 has anti-apoptotic activity on malignant cells. The effect of IL-10 is partly confined to the C-terminal domain. We examined if IT9302, an IL-10 agonist and homologous to the C-terminal domain, also is responsible for the anti-apoptotic activity of IL-10. In a study of the effect of 2.5 microg/ml of IT9302 on 3 hepato-carcinoma cell lines. HEPG2(p53 wild type), HEP3B(p53 deleted) and PLC/PRF5(p53 mutated), we saw a significant anti-proliferative effect selectively on HEPG2 (day5). In a dose-response study, (0, 0.05, 0.5 a 5 microg/ml daily for 7 days), we observed a significant inhibition of cell-proliferation ($p < 0.01$) at 5 microg/ml of IT9302. IT9302 (0, 2.5, 5 microg/ml induced IL-8 at day 2, accompanied by the induction of p53. There was no p53 induction in PLC/PRF5 cells, and the induction of IL-8 was present only in PLC/PRF5. In the IT9302 stimulated HEPG2 cells, IkappaB- α induction was

observed from day 2. In the IT9302 stimulated HEPG2 cells PARP was cleaved at day 4 and 5. In nonstimulated cells, PARP induction was evident at days 3, 4 and 5. Conclusion: The C-terminal part of IL-10 regulates cell proliferation in HEPG2 cells, a function, which may be dependent on p53 and induction of NF κ B (followed by IL-8 induction). Newly synthesized IkappaB- α , augmented by IL-10/IT9302, possibly enters the nucleus and will uncouple active NF κ B from its DNA binding site. Further, HEPG2 cells express the IL-10R2 (CRF2-4) but not the IL-10R1. We therefore speculate that the apoptotic effect of IL-10 is mediated through IL-10R2.

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The roles of Rho GTPases in chemotaxis and chemokine receptor internalization

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Rho GTPases play a very important role in cell mobility. The activation of these GTPases causes F-actin rearrangement inside the cell, and thus changes the cell morphology and drives the cell movement. The activation of Rho GTPases, RhoA, Rac1 and Cdc42, was investigated in HEK293 cells with stable expression of CXCR2 receptor. Using GST-RhoA binding domain, which only recognizes and binds to the active RhoA, the activity of RhoA was found to increase instantly after addition of ligand, MGSA or MIP-2. The maximal increase in RhoA activity was about 2-3 fold and occurred at 5-10 min after stimulation, then go back to the baseline in 30 min. Very similar responses of Cdc42 and Rac1 to the stimulation of CXCR2 ligand were obtained by GST-CRIB (Cdc42 and Rac binding domain). Although 2-3 fold of increase in GTPase activities is not striking, the active Rho GTPases may not be evenly distributed inside the cell, resulting in much large differences in localization of activated GTPase along the leading edge of the cell. This change may be sufficient to facilitate cell morphology and thus drive cell motility. This uneven distribution, or the gradient of the signal molecules formed inside the cell, may be the key for the cells to have the proper responses to a gradient, since both dominant negative and constitutive active forms of Rho GTPases attenuated CXCR2-mediated chemotaxis. It has been reported that Rho GTPases are also involved in receptor endocytosis or internal transportation in the cells. We investigated the role of Rho GTPases in the internalization of CXCR2 in HEK293 cells. We did not find any inhibition of CXCR2 internalization after the treatment of cells with toxin B, the inhibitor of all Rho GTPase subfamily members, or treatment with dominant negative forms of these GTPases. Neither did we find any significant difference in the pattern of Rho GTPase activation among cell lines expressing CXCR2 mutants defective in internalization. Thus, Rho GTPase activity is not required for internalization and CXCR2 internalization is not required for ligand activation of Rho GTPase activity in response to ligand activation of CXCR2.

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Phosphorylation Site Specific Immunoassays for quantitation of the phosphorylation and activation of human epidermal growth factor

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Epidermal Growth Factor Receptor (EGFR) belongs to the family of receptor tyrosine kinases, which regulate cell growth, survival, proliferation and differentiation. Phosphorylation plays an important role in the activation and signal transmission of EGFR. To further study this phosphorylation, we developed Phosphorylation Site-Specific (PSS) immunoassays for the detection and quantitation of these phosphorylation events of human EGFR at the pY845, pY1068, pY1086 and pY1173. This method used a pan anti-EGFR as capture antibody and individual, Phosphorylation Site-Specific Antibodies (PSSAs) as detectors. The PSS immunoassays detected in vitro auto-phosphorylated EGFR and the phosphorylated EGFR in EGF-stimulated A431 cells. They did not react with purified non-phosphorylated EGFR or unstimulated A431 cells. The specificity of the ELISAs to the individual phosphorylation site was demonstrated using phospho-peptides to block the signal. These

assays detected stimulation of EGFR in A431 cells stimulated with as little as 1 ng/mL of EGF. In comparison to western blotting, the PSS ELISA was 10-20 times more sensitive. The PSS ELISA was applied to screening of kinase inhibitors using detection of specific patterns of phosphorylation. This assay has potential for providing valuable information regarding EGFR activation status.

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IRAK Functions as A Scaffolding Protein in IL-1 Signaling Pathway

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IRAK is known to be required for the IL-1-induced activation of NF κ B and JNK. The goal of current study is to understand the molecular mechanism by which IRAK activates the intermediate proteins TRAF6, TAB2, TAK1 and TAB1. Previous study showed that the kinase activity of IRAK is not required for its function, suggesting that IRAK mediates signaling through protein-protein interaction. In this study we investigated the detail interactions of IRAK with other signaling components, proposing that IRAK functions as a scaffolding protein in the IL-1 pathway. IRAK is recruited to the IL-1 receptor upon IL-1 stimulation, where it gets hyperphosphorylated. The phosphorylated IRAK in turn recruits TRAF6 to the receptor, which is different from the previous concept that IRAK interacts with TRAF6 after it leaves the receptor. While the interaction of TRAF6 with IL-1R is transient, the interaction between IRAK and TRAF6 is significantly prolonged, suggesting that IRAK-TRAF6 dissociate from the receptor as a complex. IRAK then brings TRAF6 to interact with TAB2, TAK1 and TAB1, which are pre-associated on the membrane before stimulation. TRAF6, TAB2, TAK1 and TAB1 subsequently dissociate from the modified IRAK and translocate to the cytosol. Using the IRAK-deficient cell line, IIA, it is shown that IRAK is required for the release and translocation of TRAF6-TAB2-TAK1-TAB1 from the membrane to the cytosol. The modified IRAK remains on the membrane and eventually gets degraded. The above findings indicate that the main role of IRAK is to mediate the association and dissociation of different signaling complexes, functioning as a scaffolding protein in IL-1 signaling pathway.

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THE ROLE OF M-RAS IN THE ACTION AND PRODUCTION OF CYTOKINES

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Members of the Ras family of small GTPases play important roles in innate and adaptive immune responses. They are activated by cytokines like IL-1, IL-3, and SLF, and are required for the production of cytokines like IL-2 and the generation of B and T lymphocytes and other cells of the hemopoietic system. Most studies have focussed on p21 Ras proteins, H-, N-, and K-Ras. Recently, we described a new member of the Ras family, M Ras, which is activated by the same guanine nucleotide exchange factors as p21 Ras and shares many of the same effectors. Moreover, the tools used to study activation of p21 Ras and its function do not discriminate between p21 Ras and M-Ras. We report that M-Ras and H-Ras are differentially activated by different stimuli, M-Ras being preferentially activated by growth factors such as IL-3, whereas H-Ras was preferentially activated by ligation of the B cell antigen receptor. These differences correlated with differences in sub-cellular localization, with H-Ras, but not M-Ras colocalizing with the ligated B cell antigen receptor. Overexpression of constitutively active mutants of M-Ras or H-Ras in hemopoietic stem/progenitor cells resulted in the growth and differentiation of certain lineages of hemopoietic cells such as macrophages in the absence of stimulation by growth factors and the rapid emergence of immortalized clones which were leukemogenic in vivo. We are currently analyzing mice with targeted disruption of the M-Ras gene in terms of their ability to generate and respond to cytokines. *Supported by The CIHR*

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The mitogen-activated protein kinase MKK6 physically interacts with the double-stranded RNA-dependent protein kinase PKR

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Upon binding to double-stranded RNA (dsRNA), the protein kinase PKR is activated by autophosphorylation, and consequently phosphorylates the eukaryotic translation initiation factor eIF2- α to inhibit protein synthesis. Recently, PKR has been shown to be necessary for the p38 MAPK pathway activation in response not only to dsRNA, but also to others pro-inflammatory stimuli, which include LPS, virus and cytokines. However, the components connecting PKR to p38 activation are unknown. The major immediate activator of p38 MAPK in response to cellular stress as well as pro-inflammatory stimuli is the MAPK MKK6, which is activated through phosphorylation of serine and threonine residues at positions 207 and 211, respectively. To investigate how p38 activation is dependent on PKR, we speculated that MKK6 might be a target component for PKR. In HT1080 cells, we demonstrate that the phosphorylation of MKK6, p38 and ATF2 by dsRNA is correlated with the phosphorylation of eIF2- α , leading us to propose that PKR has a role on p38 MAPK pathway activation elicited by dsRNA. To determine whether PKR and MKK6 can interact with each other, immunoprecipitation assays were performed. We have detected physical interaction of MKK6 and catalytically inactive PKR after transient over-expression in 293T cells. We have also observed that transiently over-expressed MKK6 physically interacts with the endogenous PKR in 293T cells only after dsRNA treatment. More important, we demonstrate that PKR-MKK6 association occurs physiologically only after dsRNA treatment of HT1080 cells. Finally, PKR phosphorylates in vitro either wild-type MKK6 or mutant MKK6(S207Q, T211Q) only in the presence of dsRNA, suggesting that there are other phosphorylation sites beyond that consensus in MKK6 that might be preferred by PKR. Our results suggest that the physical association of MKK6 and PKR is substrate-kinase specific double-stranded RNA dependent, and that MKK6 might be the component linking PKR to the p38 MAPK pathway activation.

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CYTOSOLIC PHOSPHOLIPASE A2 IS ESSENTIAL FOR PROSTAGLANDINS BUT NOT FOR LEUKOTREINS FORMATION IN PHAGOCYTIC-LOKE CELLS.

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We have previously established a model of cytosolic phospholipase A2 (cPLA2)-deficient differentiated PLB-985 cells (PLB-D cells) and demonstrated that cPLA2-generated arachidonic acid (AA) is essential for NADPH oxidase activation. In the present study we used this model to determine the physiological role of cPLA2 in the production of prostaglandin E2 (PGE2) and leukotrien B4 (LTB4). Parent PLB-985 cells, and PLB-D cells were differentiated toward the monocyte- or granulocyte- lineages using 5×10^{-8} M 1,25 dihydroxyvitamin D3 (1,25(OH) $_2$ D3) or 1.25% DMSO, respectively. Parent monocytic like PLB cells released PGE2 by various agonists with the rank order of A23187 > PMA > OZ > FMLP, while monocytic like PLB-D cells did not release any PGE2 with either of the inducers. Preincubation of the cells with lipopolysaccharide (20 ng/ml) for 16 hour before stimulation enhanced PGE2 release by parent PLB cells in correlation with the increased levels of cPLA2 detected in these cells. LPS had no effect PLB-D cells. High and similar levels of LTB4 could be secreted from stimulated granulocyte-like PLB cells containing cPLA2 and PLB-D cells lacking this protein. The results of the present study indicate that cPLA2 generating AA is the major type of PLA2 responsible for the formation of PGE2 but not LTB4 in phagocytic-like cells.

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T1/ST2 signal transduction: activation of AP-1, JNK, p38 and p42/p44 MAP kinase but not NF-kappaB

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T1/ST2 is a member of the IL-1 receptor superfamily, possessing 3 immunoglobulin domains extracellularly and a Toll/IL-1 receptor domain intracellularly. It is expressed on Th2 cells and mast cells and has a role in Th2 cell effector function. The ligand for T1/ST2 is not known, and little is known about the signal transduction pathways activated by T1/ST2. We have used over-expression of T1/ST2 or a cross-linking antibody to T1/ST2 to study signal transduction. We have found that T1/ST2 does not activate NF-kappaB, but does activate AP1. T1/ST2 also activates JNK, p38 and p42/p44 MAP kinases. A chimera comprising the extracellular portion of the Type I IL-1 receptor and the intracellular portion of T1/ST2 activates NF-kappaB, but this effect is dependent upon the association of the chimera with the IL-1 receptor accessory protein, and so does not truly reflect T1/ST2 signalling. Our study therefore suggests that not all TIR domain - containing receptors signal the same, and identifies the first such receptor unable to activate NF-kB. The activation of AP1, JNK, p38 and p42/p44 MAP kinases are therefore the first signals for T1/ST2 to be reported. These results may have consequences for the role of T1/ST2 in Th2 cell function.

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SUPPRESSOR OF CYTOKINE SIGNALING (SOCS)3: ROLE IN SUPPRESSION OF MACROPHAGE ACTIVATION BY LPS.

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Interleukin 10 (IL-10) down-regulates inflammatory responses by inhibiting phagocyte activation. Previous studies have shown that IL-10 can induce the expression of the Suppressor of Cytokine Signaling (SOCS)-3 mRNA in human neutrophils and monocytes. Due to the great interest in SOCS-3, because it has the potential to negatively regulate the responses to different activating cytokines and bacterial products, it was tempting to speculate that IL-10 exerts its anti-inflammatory action by inducing SOCS-3 expression. However, direct evidence that SOCS-3 can act as the mediator of IL-10 inhibitory action on LPS-induced macrophage activation have not been provided so far. Furthermore, whether IL-10-induced SOCS-3 contributes mainly to IL-10's inhibition of the LPS response or also provides a negative feedback regulation of IL-10 signaling itself remained to be determined. In this study, we show that exogenous SOCS-3 stably transfected into the IL-10 responsive mouse macrophage cell line J774 mimics the effect of IL-10 on LPS-induced responses. Constitutive SOCS-3 expression in J774 cell line results in a reduced ability to secrete nitric oxide (NO), Tumor Necrosis Factor- α (TNF- α), Interleukin-6 (IL-6) and Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) in response to bacterial LPS. SOCS-3 overexpression reproduces IL-10 inhibitory action by modulating the inducible Nitric Oxide Synthase (iNOS) and IL-6 at the mRNA level, and by impairing the TNF- α production mainly at a post-transcriptional level. Moreover, in the presence of SOCS-3 overexpression these cells constitutively express the mRNA encoding the Interleukin-1 receptor antagonist (IL-1ra), in a manner similar to that observed in response to IL-10. Furthermore, constitutive SOCS-3 expression inhibits IL-10-induced STAT3 tyrosine phosphorylation, but does not block IL-10 inhibition of LPS-induced responses. Taken together, these data strongly indicate SOCS-3 as a key mediator of IL-10 inhibitory activities, involved in the signaling that leads to inhibition of the secretion of LPS-induced pro-inflammatory mediators.

TRANSITIONS IN INNATE AND ADAPTIVE IMMUNITY (175-177)

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Stress management by NK cells: NKG2D receptor recognition of MHC class I-like antigens on tumors and virus-infected cells

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NK cells are regulated by signals transmitted by opposing inhibitory and activating receptors. NKG2D is an activating receptor associated with the DAP10 adapter protein that is expressed on NK cells, gd-TcR+ T cells, CD8+ T cells, and activated macrophages. NKG2D recognizes two families of ligands; one encoded by the MICA and MICB genes within the human MHC and a second family of genes, RAE-1, that are present in mice and humans. The MIC and RAE-1 genes are expressed at minimal levels on normal tissues, but are over-expressed by tumors. MIC genes are induced by stress, for example heat shock and viral infection. Ectopic expression of RAE-1 on lymphomas and melanomas allows NK cells to reject these transplanted tumors in vivo. Thus, the NKG2D receptor potentially provides a mechanism of immune surveillance for cells that are abnormal, transformed, or infected by pathogens.

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The immunological synapse: coordinating T cell migration and antigen recognition

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Naive T cells patrol the secondary lymphoid tissues in a migratory circuit that is likely to allow serial interactions with thousands of antigen presenting cells. We provide in vitro evidence that antigen has the tendency to interrupt this migratory circuit by temporarily stopping T cell migration and promoting the formation of a specialized cell-cell junction we refer to as an immunological synapse. In vitro we and others can demonstrate this process using either natural antigen presenting cells or supported planar bilayers containing adhesion molecules and MHC-peptide complexes. This stop signal depends upon polarization of the T cell toward the antigen presenting surface. We have found that the immunological synapse establishes a physical environment in which additional receptor ligand interactions are fostered. For example, the interaction of CD28 and CD80 is inefficient until the formation of the immunological synapse establishes a central region in which CD28 engagement is enhanced. This finding provides a physical basis for the sequential nature of signal 1 (antigen) and signal 2 (co-stimulation). While antigen produces a stop signal, we have also found that several chemokines can produce a dominant go signal that induces T cells to break immunological synapses. Most chemokines, however, are subordinate to antigen signals such that only a subset of chemokines can negatively regulate antigen recognition. In vivo environments may change with inflammation to shift the balance in favor of the antigen stop signal or the dominant chemokine go signal.

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The role of FADD in cytokine-mediated T cell activation

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Based on the analysis of genetically deficient mice, there exists a requirement in T cell activation for signal transduction through FADD. In particular, T cells from such mice activated with anti-CD3, under conditions that require the addition of cytokines, show a severe deficiency in proliferation and differentiation. We have examined the details of this deficiency in order to understand how the canonical apoptosis pathway mediated by the FADD-activation of caspase-8 plays a role in T cell activation. Experiments examining cytokine-dependent T cell activation show that inhibition of caspase-8 has effects that closely mimic the deficiency in FADD. The effect does not appear to result from a general cell cycle inhibition since various means of generally stimulating T cells are not affected. A model of FADD-mediated intervention in cytokine signaling will be presented.

INFECTIOUS DISEASES (178)

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Role of Toll-like Receptors in Innate Immune Responses to Mycobacteria

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Mammalian Toll-like receptor (TLR) proteins are pattern recognition receptors that mediate cellular activation by a wide variety of bacterial products. TLR activation leads to the expression of pro-inflammatory cytokines, chemokines, co-stimulatory molecules, and nitric oxide by macrophages. Thus, TLR-dependent activation is likely to be required for successful immunity against bacterial pathogens. We previously showed that live *M. tuberculosis* (Mtb) bacteria can activate macrophages via both TLR2 and TLR4. We also determined whether Mtb activate macrophages via distinct TLR-dependent and TLR-independent pathways. These studies revealed that Mtb-induced TNF production was mediated by TLR proteins, but nitric oxide production appeared to be generated by a TLR independent mechanism. Interestingly, purified TLR2 and TLR4 agonists differed in their capacities to induce pro-inflammatory cytokines and nitric oxide production by macrophages in vitro. This difference suggests that intracellular signaling pathways activated by TLR2 and TLR4 are distinct, resulting in different functional responses. We subsequently sought to determine whether TLR2 and TLR4 participate in the control of mycobacterial infection in vivo. We found that knockout mice lacking TLR2 and TLR4 are more susceptible to mycobacterial infection than control mice. This susceptibility correlates with a diminished capacity to generate a potent Th1-type immunity following infection of the TLR-deficient animals. Serum levels of IL-12 and IFN-gamma in infected TLR-deficient mice were markedly lower than in infected control mice. Together, these studies indicate that TLR proteins mediate critical host responses to mycobacterial infection.

CHEMOKINES (179-181)

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CCR3 in asthma: role of the mast cell

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Asthma is a disease characterized by bronchospasm, mucus hypersecretion and non-specific airways hyperresponsiveness to smooth muscle contractile substances. An hereditary basis of a polygenic nature has been demonstrated. Most commonly, airways inflammation with eosinophils and T lymphocytes is seen microscopically. The chemokine receptor CCR3 has been implicated as a major chemoattractant receptor for Th2 lymphocytes and eosinophils and is thus in a potential position to mediate the asthmatic diathesis. Here we show that deletion of the murine CCR3 gene paradoxically is associated with the most common diagnostic sign of asthma, airways hyperresponsiveness to inhaled methacholine. We note an 8-fold increase in the number of submucosal mast cells in the knockout animals in the native state, and at least a five-fold increase in intraepithelial mast cells in the ova-sensitized lung. These data imply a role for CCR3 in mast cell trafficking and/or function. Paradoxically, mice immunized intradermally versus intraperitoneally display a decrease in airways responsiveness to methacholine after ova immunization. This is associated with a decreased number of intraepithelial mast cells. Thus, the mode of immunization allows very disparate phenotypes involving mast cells in CCR3 deficient mice.

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Chemokine-recruited DCs link inflammation and immunity

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Chemokines are a family of chemotactic cytokines. The prototypes of chemokines, interleukin 8 and MCAF/MCP-1 were discovered as chemoattractants for neutrophils and monocytes, respectively and their roles in inflammation are now well established. Recent identification of numerous lymphocyte tropic chemokines implies that chemokines are also pivotally

involved in the establishment of immune system and in regulating subset specific lymphocyte trafficking in immune responses. In this symposium, I will present our novel concept that chemokine-recruited DCs play as master cells which link inflammation and acquired immunity. In *Propionibacterium acnes* (P.acnes)-induced granulomatous liver injury in mice, CD11c+ DC precursors appeared in the circulation, migrated into the peri- sinusoidal space, and matured within newly formed granulomas. The recruited DCs later moved to the portal area to interact with T cells in portal tract-associated lymphoid tissue (PALT), and subsequently migrated into the paracortex of draining hepatic lymph nodes (LNs). MIP-1 α /CCL3 attracted blood DC precursors to the sinusoidal granuloma, while SLC/CCL21 attracted mature DCs to the PALT as well as the LNs. After arriving at the LNs, mature DCs sooner expressed MDC/CCL22 and IP-10/CXCL10 to attract nonpolarized T cells, while later preferentially produced IP-10 to promote DC-Th1 cell clusters. These Th1 polarizing lymphocytes exited the LNs, recirculated, migrated into the hepatic granulomas, and further proliferated there to complete granulomatous reactions. Thus, the function of inflammation-associated DC precursors and their trafficking regulated by chemokines are pivotal to establish Th1-mediated immune responses.

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Genetic analysis of chemokine roles in human disease

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Despite the availability of a large number of knockout mouse strains and disease models, choosing chemokine and chemokine receptor targets for therapeutic intervention in human disease remains speculative, in part because there are fundamental differences in the organization and function of the human and mouse chemokine systems. An alternative approach, which has been best validated for CCR5 in HIV/AIDS, is to search for disease associations among genetic polymorphisms that affect the function or expression of chemokines and chemokine receptors. We have analyzed variants of RANTES, CX3CR1, CCR5 and CCR2 and found several that act as independent genetic risk factors in HIV/AIDS and atherosclerotic coronary artery disease.

Additional analyses in renal transplant rejection and hepatitis C infection will be presented.

CHEMOKINES (182-189)

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Resistance to septic peritonitis in mice lacking CC chemokine receptor 8 via augmenting innate immune response

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CC chemokine receptor 8 (CCR8) is preferentially expressed by Th2 subsets and considered to play a role in Th2-type immune response. Here, we demonstrate a detrimental role of CCR8 in innate immunity during septic peritonitis. CCR8 $^{-/-}$ mice were resistant to the lethality as compared to wild-type (WT) mice, which was associated with increased bacterial clearance in CCR8 $^{-/-}$ mice. CCR8 expression was augmented in peritoneal macrophages during sepsis. In vitro, CCR8 $^{-/-}$ macrophages cleared more bacteria than WT macrophages. CCR8 $^{-/-}$ macrophages produced significantly higher levels of superoxide generation, lysosomal enzyme release and nitric oxide production, as compared to WT macrophages, upon stimulation with LPS. Levels of thymus-derived chemotactic agent 3 (TCA3; CCL1) and thymus and activation-regulated chemokine (TARC; CCL17), ligands for CCR8, were quantitated in the peritoneum, which showed that TARC, but not TCA3, was elevated during septic peritonitis. Neutralization of TARC with anti-TARC antibodies resulted in a decreased bacterial load in the peritoneum. The event was accompanied by increases in the peritoneal levels of TNF α , MIP-2, KC and MCP-1, all of which were known to enhance bacterial clearance. Thus,

CCR8/TARC appears to be deleterious in host defense during septic peritonitis via altering the innate immune response.

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Chemokine Receptor Polymorphism and Risk of Acute Rejection in Human Renal Transplantation

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Chemokines regulate the trafficking of leukocytes in immunity and inflammation and have been implicated in mouse models in acute cardiac and renal allograft rejection; however, the significance of this to human transplantation is not yet defined. Here we examined the association of human chemokine receptor genetic variants, CCR5- Δ 32, CCR5-59029-A/G, CCR2-V64I, CX3CR1-V249I and CX3CR1-T280M, with outcome in 166 renal transplant recipients. We found significant reductions in risk of acute renal transplant rejection in recipients who possessed the CCR2-64I allele (Odds Ratio 0.30; 95% Confidence Interval 0.12-0.78; $p=0.014$) or were homozygous for the 59029-A allele (Odds Ratio 0.37; 95% Confidence Interval 0.16-0.85; $p=0.016$). There was no significant difference in the incidence of rejection among patients stratified as with or without CCR5- Δ 32, or by the CX3CR1-V249I, or CX3CR1-T280M genotypes. Multivariate analysis including sex, race, age of the donors and recipients, HLA-A, B, DR mismatch, previous transplant, type of transplant (cadaveric vs. living), immunosuppression, and delayed graft function confirmed the univariate findings for possession of the CCR2-64I allele (Odds Ratio 0.18, $p=0.026$) and homozygosity for the 59029-A allele (Odds Ratio 0.31, $p=0.037$). We conclude that the risk of acute rejection in renal transplantation is associated with genetic variation in the chemokine receptors CCR2 and CCR5.

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Hsc70 interacting protein (Hip) associates with CXCR2 and regulates the receptor signaling and trafficking

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The Hsc70-interacting protein Hip, a tetratricopeptide repeat (TPR) protein, participates in the regulation of the eukaryotic 70 kDa heat shock cognate Hsc70, a chaperone that plays an important role in protein assembly and trafficking. Hip stabilizes the ADP state of Hsc70 that has a high affinity for substrate proteins. Through its own chaperone activity, Hip may contribute to the interaction of Hsc70 with various target proteins. In an effort to identify intracellular proteins that interact with the G protein-coupled chemokine receptor CXCR2 using yeast-two hybrid screening, Hip was found to be of interest. In the present study we demonstrate the formation of an agonist-induced complex containing CXCR2 and Hip. Hip binds through its carboxyl terminus to the C-terminal leucine-rich domain of CXCR2 in GST-pull down experiments. Agonist treatment induces a time-dependent association of CXCR2 with Hip in RBL-2H3 cells, and the agonist-dependent association is blocked by mutation of IL323,324 motif in the receptor C-terminus. Overexpression of a TPR deletion mutant of Hip (DTPR), which loses the Hsc70 binding domain but is able to bind to CXCR2, attenuates agonist-induced internalization of CXCR2, without affecting the receptor expression on the cell surface and the receptor-ligand binding. Furthermore, CXCR2-mediated chemotaxis is blocked by overexpression of DTPR in RBL-2H3 cells. Our data indicate for the first time that Hip plays an essential role in chemokine receptor endocytosis and in chemokine receptor-mediated chemotaxis. We postulate that the effects of Hip involve Hsc70, which has been demonstrated to mediate the internalization and recycling of other membrane proteins other than G protein-coupled receptors through affecting the cycling of clathrin-coated vesicles.

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Induction of chemokine CXCL11/ β -R1 by IFN- β requires PI3K

M. R. Sandhya Rani*, Nywana Sizemore*, George R. Stark* and Richard M. Ransohoff**. Dept. of **Neurosciences and *Molecular Biology, Lerner Research Institute, The Cleveland Clinic Foundation, Cleveland, OH 44195. The β -R1 (SCYB11) gene encodes an α -chemokine (I-TAC/CXCL11) that is a potent chemoattractant for activated T-cells. Induction of β -R1 by IFN- β required transcription factors ISGF-3 and NF- κ B. IFN- β treatment did not induce nuclear translocation or DNA-binding activity of NF- κ B. However, nuclear extracts of IFN- β treated cells contained an activity that markedly enhanced phosphorylation of the GST-fused carboxy terminal transactivation domain of p65. It was proposed that IFN- β -mediated enhancement of the transactivation competence of NF- κ B was essential for transcription of β -R1. We now present evidence for the involvement of PI3K in this pathway. IFN- β treatment induced PI lipid kinase activity, which co-precipitated with IFNAR1 and also resulted in the activation of AKT. Pretreatment of HT1080-derived fibrosarcoma cells with chemical inhibitors to PI3K (Wortmannin or LY294002) selectively inhibited β -R1 mRNA accumulation. Interestingly, experiments using PTEN (phosphatase and tensin homologue mutated on chromosome ten) mutants suggested that the lipid kinase activity of PI3K was essential for IFN- β -induced transcription of β -R1. IFN- β -mediated phosphorylation of GST-p65 was blocked by pretreatment with LY294002, suggesting that IFN- β acts through PI3K to enhance the transactivation competence of pre-existing NF- κ B complexes through phosphorylation of p65. With ISGF-3, activated NF- κ B complexes drive IFN- β -mediated transcription of β -R1. (Supported by PO1 CA6220)

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Interleukin-8-mediated angiogenesis by enhanced endothelial cell survival, proliferation and up-regulation of MMPs production.

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Interleukin-8 (IL-8), a multifunctional chemokine, has been shown to induce angiogenesis and promote tumor growth and metastasis. To determine the mechanism of IL-8-mediated angiogenesis, we examined the expression of CXCR1 and CXCR2 and effect of IL-8 in modulating endothelial cell proliferation, survival and production of matrix metalloproteinases (MMPs) in human umbilical vein endothelial cells (HUVEC). We found constitutive steady state mRNA and protein expression of IL-8 receptors CXCR1 and CXCR2 in HUVEC from two different sources. Recombinant human IL-8 induced HUVEC proliferation and capillary tube organization in a concentration-dependent manner. Neutralizing antibody to IL-8 inhibited the induction of IL-8-mediated endothelial cells capillary tube organization. The addition of exogenous IL-8 enhanced the mRNA levels of the anti-apoptotic genes. An increased bcl-xL level led to a highly elevated ratio of bcl-xL to bcl-xS. Similarly, an increased ratio of bcl-2 to bax was also observed in HUVEC treated with IL-8. Neutralizing antibodies to IL-8, CXCR1 and CXCR2 inhibited HUVEC proliferation. Further, treatment of HUVEC with IL-8 up-regulated MMP-2 and MMP-9 mRNA and activity, in contrast, HUVEC treated with neutralizing antibodies to IL-8, CXCR1 and CXCR2 down-regulated MMP-2 activity. Our results demonstrate a direct role of IL-8 in angiogenesis by interacting through its own receptors, enhancing endothelial cell proliferation, survival and up-regulation of MMPs expression.

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TNF- α mediates SDF-1 α -induced NF- κ B activation, chemokine induction and cytotoxic effects in primary astrocytes

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SDF-1 α (CXCL12) and its receptor, CXCR4 are constitutively expressed on

neuroepithelial cells and are believed to be involved in pathological processes such as AIDS-associated neurologic disorders as well as development. In the present study, we demonstrate that SDF-1 α activates NF- κ B, stimulates production of chemokines and cytokines and induces cell death in primary astrocytes, effects that depend on ongoing secretion of TNF- α . SDF-1 α upregulated TNF- α mRNA and protein secretion as well as TNF receptor 2 expression. TNF- α treatment mimicked SDF-1 α induction of NF- κ B, IL-1 α / β and RANTES, and cell death; neutralizing antibodies against TNF- α opposed these responses. We also found that SDF-1 α activated Erk1/2 MAPK in a biphasic fashion. Early Erk1/2 activation was stimulated directly by SDF-1 α and late activation was mediated by TNF- α . PD98059 suppression of early Erk1/2 activation correlated with reduction of SDF-1 α -induced TNF- α expression. Late Erk1/2 activation was involved in TNF- α -stimulated NF- κ B activation, cytokine induction. SDF-1 α was induced in reactive CXCR4-positive astrocytes near axotomized spinal cord motor neurons. We propose that these novel effects of SDF-1 α are relevant to the pathogenic and developmental roles of SDF-1 α in the CNS.

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ANTIBACTERIAL PEPTIDES HUMAN β -DEFENSIN-2 AND LL-37 ACT AS CHEMOTACTIC FACTORS FOR MAST CELLS THROUGH DISTINCT RECEPTORS MEDIATING PERTUSSIS TOXIN-SENSITIVE SIGNALING

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In humans, mast cells accumulate in the inflammatory conditions such as allergic reactions, in response to local chemotactic factors. Since human β -defensin-2 (hBD-2) and LL-37 are generated in the epithelial tissues where mast cells are present, and because we have recently reported that these peptides induce mast cell degranulation, we hypothesized that they could chemoattract mast cells. The present study has shown that both hBD-2 and LL-37 directly and specifically induce mast cell migration, and hBD-2 is more potent than LL-37. Checkerboard analysis showed that this migration is more chemotactic rather than chemokinetic. Moreover, Scatchard analyses using 125 I-labeled hBD-2 and 125 I-labeled LL-37-derived peptide revealed that mast cells express two classes of receptors, high- and low-affinity receptors, for both hBD-2 and LL-37. Furthermore, the competitive binding assay as well as Ca^{2+} transient desensitization experiment suggested that hBD-2 and LL-37 unlikely share the same receptors on mast cells. The treatment of cells with G protein inhibitor, pertussis toxin, and phospholipase C inhibitor, U-73122, abolished the mast cell chemotaxis towards hBD-2 and LL-37, suggesting the involvement of G protein-phospholipase C signaling pathway in the mast cell activation. Thus, antibacterial peptides β -defensins and LL-37, which are originally believed to be involved in innate host defense, may also participate in inflammatory responses through the recruitment of mast cells to inflammation foci.

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INDUCTION OF NEUTROPHIL IL-8 BY FIBRINOGEN AND fMLF OR LTB $_4$

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During diapedesis, IL-8 in exudative neutrophils is elevated > 100-fold compared to peripheral blood neutrophils but the mechanism for this increase is poorly understood. Treatment of peripheral blood neutrophils with a physiologic dose of fibrinogen prior to the addition of a chemotactic dose of either fMLF or LTB $_4$, results in synergistic induction of IL-8 to the level observed in exudative cells. Induction of IL-8 is chemoattractant specific; C5a, PAF, and GRO- α fail to mimic the effect observed with fMLF or LTB $_4$. Synergistic induction of IL-8 observed with Fib+fMLF and Fib+LTB $_4$ are sensitive to specific receptor antagonists, N-t-BOC-MetLeuPhe and LTB $_4$ dimethylamine, respectively, and to pertussis toxin, and are therefore mediated

by their respective G protein-coupled receptors. The observed synergy is also specific for fibrinogen. Heat-treated fibrinogen, fibrinopeptides A/B, fibrin, as well as extracellular matrix proteins (vitronectin, fibronectin, laminin, collagen, and thrombospondin) and acute phase proteins (serum amyloid A, transferrin, etc.) fail to exhibit synergy with fMLF. Analysis of fibrinogen by gel filtration chromatography results in a single peak of IL-8-inducing activity that co-elutes with fibrinogen. Removal of the fibrinogen by treatment with thrombin abrogates the IL-8-inducing activity. Finally, recombinant fibrinogen mimics the effect of native fibrinogen in inducing neutrophil IL-8 production. The production of IL-8 in vitro by neutrophils mimics that observed in exudative neutrophils and indicates that migrating neutrophils are a potential source of IL-8 found at inflammatory foci.

INNATE IMMUNITY (190-197)

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Identification of a Molecule Critical for Lymphocyte Motility

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The immune system is an indispensable self-defense mechanism that has been evolved through the interaction with various microorganisms such as bacteria, viruses and parasites. To cope with the infection of such microorganisms, cells in the immune system are equipped with highly motile property. While macrophages and neutrophils rapidly migrate to the lesion to function in the initial defense, T cells and B cells differentiated in the primary lymphoid organs migrate to particular sites of the secondary lymphoid organs to achieve normal architecture of the immune system and provide the 'place' for proper immune response. Recent advance in chemokines and their receptors have revealed that different chemokines lead particular types of cells to the specific compartments of the immune system. However, the molecular basis underlying this polarized cell migration is poorly understood. We have identified a molecule that regulates remodeling of actin cytoskeleton and critically affects lymphocyte motility. Based on the findings of knock out mice, we will discuss about the role of this molecule in the architecture and function of the immune system.

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Particulates can induce macrophage lineage survival and proliferation: implications for the chronicity of inflammatory responses and adjuvant action.

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One possible mechanism for the increased numbers of macrophage lineage cells at sites of inflammation and immune reactions is increased survival and even local proliferation. Macrophage lineage cells, such as murine bone marrow-derived macrophages (BMM), are dependent upon the growth factor, macrophage colony stimulating factor (M-CSF or CSF-1), for their survival and proliferation. Certain particulates are poorly degraded by macrophages and therefore can persist inside them. We determined whether such particulates could promote survival and possibly proliferation in this lineage. We found that BMM survival was enhanced after treatment with the following poorly degradable particles: oxidized low density lipoprotein, adjuvants (alum, oil in water emulsions, heat-killed bacteria, silica, methylated BSA), crystals (basic calcium phosphate, monosodium urate, talc), and amyloidogenic peptides (amyloid beta and prion protein). They could also induce DNA synthesis, particularly in the presence of suboptimal (circulating) concentrations of CSF-1. Both responses were independent of endogenous CSF-1 or granulocyte macrophage-CSF (GM-CSF) since BMM from op/op/GM-CSF^{-/-} mice responded normally. Human monocytes also showed increased survival. It is proposed that this enhanced survival and even proliferative response occurring when macrophage lineage cells confront certain poorly degradable particulates could in part lead to persistence of an inflammatory response and help to explain the action of particulate adjuvants.

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RELB REGULATES THE DIFFERENTIATION OF INNATE IMMUNE CELLS

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RelB, a member of the Rel/NF-kappaB family of transcription factors, is required for the development of myeloid dendritic cells, but its role in lymphocyte development is less well characterized. Here we report selective defects in both homozygous RelB knockout and RelB heterozygous mice in the development of specialized lymphocyte populations, in particular NK T cells and intestinal epithelial lymphocytes (IEL). However, conventional T cell numbers were not reduced. Interestingly, while NK cell numbers were normal, their function was partially impaired. Transfer of bone marrow from RelB knockout mice into RAG2 deficient recipients restored both IEL and NK T cells, indicating that a RelB expressing nonlymphoid cell is indispensable for their development. In additional experiments however, we demonstrated that development of CD8alpha+ IELs required the presence of beta-2 microglobulin on this radio-resistant host cell, whereas the development of NK T cells did not. These data demonstrate new requirements for the development of NK T cells and IEL and indicate a nonredundant role for RelB in natural lymphocytes that bridge innate and adaptive immunity.

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Granulocyte colony stimulating factor (G-CSF) promotes firm adhesion of PMN to endothelial cells

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G-CSF as a cytokine responsible for PMN maturation and mobilization may play a role in PMN recruitment at sites of inflammation. However, molecular events possibly relevant to inflammatory processes that occur during G-CSF-mediated PMN stimulation are unknown. We investigated the ability of G-CSF to alter PMN adhesion in two models each utilizing mature human PMN isolated from peripheral blood. The first model assessed adhesion under conditions of shear and static to target cells transfected with human ICAM-1, and the second involved adhesion to cytokine-activated human umbilical vein endothelial cells (HUVEC). Under static and low shearing conditions G-CSF induced significant PMN adhesion to ICAM-1 expressing cells. G-CSF promotes this adhesion by activating both Mac-1 and LFA-1 in PMN. Under both shearing and static conditions G-CSF-mediated PMN adhesion to ICAM-1 peaks at 11 minutes without detectable surface upregulation of Mac-1. This is in marked contrast to chemokine stimulation, which peaks within 1-2 minutes after stimulation. G-CSF-mediated PMN adhesion was unaffected by pertussis toxin, indicating that G-CSF activation of Mac-1 and LFA-1 is not indirectly mediated by chemokine receptors. G-CSF also induced PMN adhesion to IL-1 stimulated HUVEC and G-CSF promoted this adhesion through activation of PMN adhesive mechanisms since HUVEC lack functional G-CSF receptors. These results indicate that G-CSF promotes firm adhesion of PMN.

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ULBPS, NOVEL MHC CLASS I-RELATED PROTEINS, BIND TO NKG2D AND ACTIVATE MULTIPLE SIGNALING PATHWAYS IN PRIMARY NK CELLS

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The ULBPs are a novel family of GPI-linked, MHC class I-related molecules that were identified as targets of the human cytomegalovirus glycoprotein, UL16. We have previously shown that ULBPs bind to NK cells and generate a stimulatory signal that can override negative signals generated by engagement

of inhibitory MHC class I receptors. Here we show that NKG2D, an activating receptor expressed by NK and other immune effector cells, is the ULBP counterstructure on primary human NK cells, and that its expression is highly upregulated by IL-15. By binding NKG2D, soluble forms of ULBPs induce calcium mobilization, marked protein tyrosine phosphorylation, and activation of the JAK2, STAT5, ERK MAP kinase and Akt/PKB signal transduction pathways in primary NK cells. ULBP-induced activation of Akt and ERK and ULBP-induced IFN- γ production are blocked by inhibitors of PI 3-kinase. These findings are consistent with the known binding of PI 3-kinase to DAP10; the membrane bound signal transducing subunit of the NKG2D receptor. While all three ULBPs activate the same signaling pathways, ULBP3 was found to bind weakly and to induce the weakest signal. Finally, we found that ULBP messages are expressed by a wide range of cells, tissues and tumors and that ULBP proteins are expressed in various cell lines. Thus, several types of cells may potentially deliver ULBP-mediated signals to NK cells and be targets of ULBP-mediated killing. In summary, we have shown that NKG2D is the ULBP counterstructure on primary NK cells and for the first time have identified signaling pathways that are activated by NKG2D ligands. These results increase our understanding of the mechanisms by which ULBP/NKG2D interactions activate immune effector cells, and may have implications for immune surveillance against pathogens and tumors.

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TOLL-LIKE RECEPTOR 2 (TLR2) AND TLR4 DIFFERENTIALLY ACTIVATE HUMAN DENDRITIC CELLS

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Toll-like receptors (TLR) mediate cell activation by various microbial products. Here we demonstrate that activation of DC by TLR2 or TLR4 agonists, although leading to comparable activation of NF- κ B and MAPK family members, resulted in striking differences in cytokine and chemokine gene transcription, suggesting that TLR2 and TLR4 signaling is not equivalent. A TLR4 agonist specifically promoted the production of the Th1 inducing cytokine IL-12 p70 and the chemokine IP-10, which is also associated to Th1 responses. In contrast, TLR2 stimulation failed to induce IL-12 p70 and IP-10, but resulted in the release of the IL-12 inhibitory p40 homodimer, producing conditions that are predicted to favor Th2 development. TLR2 stimulation also resulted in preferential induction of IL-8 and p19/IL-23. Involvement of PI3-Kinase and p38 MAP Kinase in the TLR-mediated induction of several cytokines and chemokines messages was demonstrated using specific inhibitors. Thus, TLRs can translate the information regarding the nature of pathogens into differences in the cytokines and chemokines produced by DC and therefore contribute to the polarization of the acquired immune response.

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Regulation of neutrophil production in adhesion molecule-deficient mice by IL-17 and G-CSF

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Most mutant mice lacking leukocyte adhesion molecules have moderate to severe neutrophilia. To investigate whether neutrophilia may result from an altered endogenous feedback loop, we generated chimeric mice by transplanting lethally irradiated wild-type mice with CD18 $^{+/+}$ and CD18 $^{-/-}$ bone marrow cells at various ratios. The resulting CD18-expressing population in blood neutrophils corresponded to the proportion of CD18 $^{+/+}$ bone marrow. Even at 10% CD18 $^{+/+}$ neutrophils, the neutrophilia typical of CD18 $^{-/-}$ mice was completely reversed. These data show that neutrophilia is not a result of enhanced neutrophil survival or their passive accumulation because they cannot leave the circulation. Next, we measured IL-17 and G-CSF levels in these and other adhesion molecule-deficient mice and found an elevation of both cytokines that was in proportion to the neutrophilia seen. Blocking IL-17 reduced both G-CSF and neutrophil counts, while blocking G-CSF reduced

neutrophil counts only and did not change IL-17, suggesting that IL-17 is upstream from G-CSF. We conclude that blood neutrophil counts are regulated by a feedback loop involving G-CSF and IL-17, and that this feedback loop is disrupted when neutrophils cannot migrate properly into their target tissues.

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CD14 and Toll 4 play no role in the response to encapsulated Gram-negative bacteria

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Severe infection by Gram-negative bacteria can lead to death due to septic shock. Injection of LPS (lipopolysaccharide, endotoxin), a component of the outer membrane of Gram-negative bacteria, into animals induces shock-like symptoms and death. We have previously shown that mice deficient in the CD14 LPS receptor are resistant to the lethal effects of both LPS and live *E. coli* 0111, a non-encapsulated Gram-negative bacterium. However, since some strains of Gram-negative bacteria isolated from septic patients bear a capsule that covers the LPS, we examined the role of CD14 in the response to encapsulated bacteria. Thus, CD14 $^{-/-}$ or CD14 $^{+/+}$ mice were injected with different strains of encapsulated (K1) or non-encapsulated *E. coli* obtained from sepsis patients and their response was analyzed by measuring survival and TNF α production. The CD14 $^{-/-}$ mice were resistant to a dose of non-encapsulated *E. coli* which was lethal for CD14 $^{+/+}$ mice and produced at least 10-fold less TNF α than CD14 $^{+/+}$ mice, as previously seen with *E. coli* 0111. In contrast, CD14 $^{-/-}$ mice were as sensitive to a lethal injection of encapsulated *E. coli* as CD14 $^{+/+}$ mice, even though plasma levels of TNF α in CD14 $^{-/-}$ mice were at least 5 to 10 fold lower than in CD14 $^{+/+}$ mice. Similar observations were made with mice deficient in Tlr4, a critical component of the LPS receptor signaling complex. These results support the conclusion that CD14 and Toll 4 play no role in the lethality induced by encapsulated Gram-negative bacteria.

INFECTIOUS DISEASES (198-205)

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DIFFERENTIAL SIGNALING AND REPLICATION OF HIV STRAINS Infecting T Lymphocytes via CCR5, CXCR4, or both Co-receptors

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The discovery of the precise mechanism of infection by the human immunodeficiency virus type-1 (HIV-1) via interaction with CD4 and a selected chemokine receptors has shed new lights and posed new questions on the intimate mechanisms leading to pathogenesis and viral replication. Paradoxically, viral strains with the most virulent phenotype in vitro, i.e. those using CXCR4 (X4 viruses) as entry co-receptor instead of CCR5 (R5 viruses), are not favored during vertical or horizontal transmission in vivo. We have utilized different models, including Th1, Th2 or unpolarized (Th0) T cell clones or cord blood T cell lines (CB lines), of in vitro infection by laboratory-adapted and primary HIV-1 strains with differential usage of chemokine receptors (CCR5, CCR3, CXCR4). R5 viruses efficiently replicated in primary T cells in the presence of interleukin-2 (IL-2) also when infected 15 days or more after their initial stimulation by anti-CD3 monoclonal antibodies (mAb) regardless of their state of functional polarization. In contrast, X4 strains did not replicate efficiently in these cells unless they were re-stimulated by anti-CD3 mAb. Noteworthy, R5 HIV replication was not affected by anti-CD3 mAb re-stimulation suggesting that signaling via CCR5 (either alone or together with CD4) may substitute for mitogenic activation. Primary dualtropic (R5/X4, R3/X4) HIV isolates, typically emerging before the onset of the clinical symptoms of AIDS in about 50% of individuals infected by clade B HIV-1, efficiently replicated in Th0 and Th2, but not in Th1 cells. Therefore, R5 viruses, that sustain the AIDS pandemic and are the only viruses efficiently transmitted from one infected person to another, display a selective replicative advantage over X4 strains at a post-entry level and likely involving differential signaling through the chemokine receptor, an hypothesis that we

are investigating by microarray analysis. In addition, Th2 polarization of CD4+ T lymphocytes may favor the emergence of dualtropic HIVs, typically observed in advanced stages of HIV disease.

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LYMPHOTOXINS CONTROL CYTOMEGALOVIRUS THROUGH REGULATION OF INTERFERON GENE EXPRESSION, AN EXAMPLE OF HOST-VIRUS DÉTENTE.

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The anti-viral effect of tumor necrosis factor (TNF) is well documented, and it has been suggested that TNF functions by mediating apoptosis of infected cells. Lymphotoxins (LT) α and LT β , members of the TNF superfamily, signal through TNF receptor (TNFR) or the lymphotoxin beta receptor (LT β R), respectively. We have identified a potent antiviral activity induced by these cytokines that specifically suppresses the replication of human cytomegalovirus (HCMV) in primary fibroblasts. Activation of TNFR or LT β R by cytokines or agonistic antibodies completely blocks HCMV spread at a late stage in viral replication, and this block is reversible, indicating a non-apoptotic mode of action for LT. LT anti-viral activity is suppressed by a dominant negative mutant of I κ B α , but not FADD or TRAF3, further supporting a non-apoptotic mechanism. The NF κ B-dependent, antiviral activity of LT is dependant upon induction of interferon beta (IFN β), and both HCMV infection and LT are required to efficiently activate IFN β transcription (> 100 fold higher induction of IFN β with addition of LT). LT α deficient mice are profoundly susceptible to murine CMV infection (LD50 of 2x10⁴ pfu vs. 2x10⁶), and mice which constitutively express a soluble LT β R:Fc "decoy" receptor are also more susceptible to MCMV, indicating a specific role for LT in control of CMV replication *in vivo*. Based on these data, we speculate that LT could be a key cytokine in the control of CMV replication and persistence.

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Stimulation via CD40 can substitute for CD4 T cell function in preventing reactivation of a latent herpesvirus.

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Reactivation of latent herpesviruses is a particular problem in immunocompromised individuals, such as AIDS patients, who lack effective CD4 T helper cell function. An important question is whether residual immune defenses can be mobilized to combat such opportunistic infections, in the absence of CD4 T cells. In the present study, we used a mouse model of opportunistic infection to determine whether stimulation via CD40 could substitute for CD4 T cell function in preventing reactivation of a latent herpesvirus. Treatment with an agonistic antibody to CD40 was highly effective in preventing reactivation of latent murine gammaherpesvirus (MHV-68) in the lungs of CD4 T cell deficient mice. CD8+ T cells were essential for this effect, whereas virus-specific serum antibody was undetectable and IFN- γ production was unchanged. This demonstration that immunostimulation via CD40 can replace CD4 T cell help in controlling latent virus *in vivo* has potential implications for the development of novel therapeutic agents to prevent viral reactivation in immunocompromised patients.

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IFN- β IS CRITICAL FOR A HOST IMMUNE RESPONSE TO VIRAL OR TUMOR CHALLENGE

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Insights into functions of the IFN- α/β system have been provided from studies with IFNAR-/- mice. Since IFN- α s and IFN- β activate the same receptor, distinctions amongst IFNs in the context of biological activities cannot be evaluated in IFNAR-/- mice. Accordingly, we generated mice null for IFN- β . Comprehensive tissue characterization has revealed no overt phenotypical changes in adult IFN- β -/- mice. Examination of PB, thymus, BM and spleen indicated no abnormalities of T cell lineages. However, BM derived B cells exhibit a potential defect in maturation, associated with a decrease in B220+ve/high/CD43^{low} cells and reduction in IgM surface expression. Additionally, we observe decreased levels of circulating Mac-1+ and Gr-1+ cells in IFN β -/- mice. Moreover, tumor growth in mice inoculated with Lewis lung carcinoma is more aggressive in IFN- β null mice. These mice are unable to attain the levels of circulating T cells observed in wildtype mice, suggestive of a limited T cell response. Anomalies in macrophage phagocytosis and NK cell functions are also observed in IFN- β -/- mice. The group B coxsackieviruses (CVB) are established agents of human myocarditis. In mice, protection from cardioselective infection with CVB3 requires intact Type I IFN signaling, though specific roles for IFN- α s versus IFN- β are unknown. Our studies with a cardiovirulent strain of CVB3 reveal an increased susceptibility and high mortality in IFN- β -/- mice. Infected heart tissue derived from IFN- β -/- mice exhibit T cell and macrophage infiltration. Differences in the extent of infection in liver, heart, spleen and brain were observed, suggestive of a distinct role for IFN- β in tissue-restricted antiviral mechanisms.

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The cytokine inducer Murabutide inhibits the expression of a novel cellular RNA helicase necessary for HIV replication

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The synthetic immunomodulator Murabutide induces chemokine release and inhibits HIV replication by multiple pathways in different cell populations. This study was designed to elucidate the molecular mechanism of the virus-suppressive activity of Murabutide. Differential display (DD)-RT-PCR analysis of regulated genes in infected lymphocytes, after treatment with Murabutide, revealed the inhibited expression of a gene with unmatched sequence identity. Full-length cloning of the respective cDNA identified the gene as a new member of the DEh RNA helicases and was named RH116. Using Hela-CD4 cells as HIV-1 infection model, transfection of the RH116 cDNA resulted in the up-regulation of viral p24 release and in the enhanced expression of unspliced and singly spliced viral mRNA transcripts. Furthermore, the RH116 protein was also found to be a target of autoimmune responses in HIV-1 patients and autoantibodies to RH116 persisted despite efficacious antiretroviral therapy. These findings implicate a novel putative RNA helicase in the cell-virus interaction and reveal a potential value for immunomodulation in the control of viral replication.

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Signaling Pathways and Gene Expression in HIV-1 Infected Macrophages

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HIV-1 infection of CD4+ T lymphocytes leads to their death and progressive loss, whereas HIV-1-infected macrophages appear to resist HIV-1 mediated apoptotic death. The differential response of these two host cell populations may be critical in the development of immunodeficiency and long term persistence of the virus. To characterize how HIV-1 modulates macrophage cellular functions without driving their demise, isolated human monocyte-

derived macrophages were exposed to an M-tropic (R5) strain of HIV-1 for different time intervals, and their molecular and functional responses monitored. Exposure of macrophages to HIV-1(BaL) resulted in rapid phosphorylation of Pyk2 and p38 MAPK. Triggering of this signaling cascade led to transcriptional regulation of multiple genes including those associated with host defense, cell cycle, NF κ B regulation and apoptosis. The enhanced gene expression was transient, declining to near control levels within 24 hrs. During this quiescent state, the virus appears to continue its life cycle unimpeded. By day 7-10 when viral replication becomes prominent as monitored by p24 antigen and electron microscopy, a number of increased genes become detected in the macrophage host. The cyclin dependent kinase inhibitor (CDKN1A) p21, MCP-1 and p38 MAPK were amongst the genes that were consistently upregulated. Induction of p38 MAPK and MCP-1 may generate a permissive environment for viral replication and facilitate viral transmission to new viral hosts. CDKN1A may favor macrophage quiescence by promoting differentiation and DNA repair in the host cells, in this way protecting macrophages against DNA damage and death.

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A NEW STRATEGY IS NEEDED TO CURE CHRONIC HIV INFECTION

Kendall A. Smith, David Warren, Paul Bellman, Ann Marie Dunne, and Maria Lobo. Weill Medical College of Cornell University, New York, NY 10021 Although antiviral drugs are effective in suppressing replication of the Human Immunodeficiency Virus (HIV), they cannot cure the infection. Therefore, we have developed a new strategy, to combine immunotherapy with antiviral therapy, in hopes of augmenting immune reactivity to HIV so that antiviral drugs are no longer necessary. In a preliminary study of 16 chronically infected subjects, antiviral therapy was interrupted while low dose daily interleukin 2 (IL2) therapy was continued. Upon relapse of viremia there was a readily detectable in vivo host antiviral response: the CD8+ T cell concentration doubled coincident with a 10-fold decline in plasma [HIV]. The rate and magnitude of decline in plasma HIV correlated with the magnitude of the CD8+ lymphocytosis. These data support the interpretation that 2 signals are necessary to maximally activate the antiviral immune response, HIV antigens and IL2. Therefore, we have initiated a phase II randomized controlled trial to test the hypothesis that a therapeutic HIV vaccine (canarypox vCP1452) and low dose daily IL2 therapy will maximally boost antiviral immune reactivity and allow the discontinuation of antiviral drugs. Thus far, 15 of 92 subjects have enrolled for a 3 month immunotherapy step, followed by a 3 month Diagnostic Treatment Interruption (DTI). The in vivo host antiviral immune response monitored by viral and lymphocyte dynamics will be correlated with enumeration of in vitro HIV specific CD4+ and CD8+ T cells.

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Experimental African Trypanosomiasis: Interleukin-10 is Crucial for Survival

Henry Tabel, Menging Shi and Wanling Pan. Dept. of Veterinary Microbiology, University of Saskatchewan, Saskatoon SK S7N 5B4, Canada We have previously shown that IFN- γ and IL-10 are involved in the development of immunosuppression in BALB/c mice infected with *Trypanosoma congolense* (ParasiteImmunol.20: 239.1998; J. Immunol.161:5516.1998) and that excessively high levels of IFN- γ are mediating the death of these mice (J. Immunol.161:5507.1998). Administration of anti-IL-10 antibodies in vivo prolonged the survival of T. congolense-infected BALB/c mice moderately (Parasite Immunol. 20:239.1998). In contrast, we demonstrate, in this study, that administration of monoclonal antibodies against murine IL-10 receptor (anti-IL-10R) not only shortened the survival time of highly susceptible T. congolense-infected BALB/c mice, but also that of relatively resistant T. congolense-infected C57BL/6 mice. The acute death of T. congolense-infected BALB/c and C57BL/6 mice treated with anti-IL-10R antibodies in vivo was associated with significantly increased plasma levels of IL-6, IL-10, IL-12 and IFN- γ . Furthermore, the production of IL-6, IL-10, IL-12 and IFN- γ by plastic-adherent spleen cells of T. congolense-infected BALB/c mice was significantly

enhanced by treatment with anti-IL-10R in vitro. These results strongly suggest that the presence and unimpaired function of IL-10 is an absolute requirement for the survival of T. congolense-infected mice. We conclude that IL-10 has detrimental as well as beneficial effects in T. congolense infections. We speculate that one of the major beneficial effects of IL-10 in T. congolense infections is the down-regulation of overproduction of IFN- γ and prevention of lethal effects of IFN- γ .

GENETIC DETERMINANTS OF HOST RESISTANCE (206)

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DISTRIBUTION OF INDUCIBLE NITRIC OXIDE SYNTHASE (NOS2) HAPLOTYPES IN U.S. BEEF CATTLE.

C.G. Chitko-McKown, W.W. Laegreid, and M.P. Heaton. USDA, ARS, U.S. Meat Animal Research Center (MARC), Clay Center, NE 68933-0166. Nitric oxide (NO), an important component of the innate immune response, is derived from L-arginine in a reaction catalyzed by nitric oxide synthase (NOS). Although constitutive expression of NOS proteins occurs in various cells, professional phagocytes such as macrophages contain an inducible form of this enzyme (iNOS or NOS2). Induction of NOS2 requires exogenous stimulation of the macrophage by factors such as IFN- γ and LPS, and results in the production of high concentrations of reactive nitrogen intermediates (ROI) and subsequent microbial killing. We identified 9 SNPs within the bovine NOS2 (bNOS2) gene by sequencing PCR amplification products of a portion of the gene homologous to human NOS2 intron 7, using genomic DNA from a panel of 96 sires from 17 popular breeds of U.S. beef cattle. Polymorphisms were verified by MALDI-TOF mass spectrometry as well as by segregation in the MARC reference population. Four SNPs were further analyzed for haplotype structure in U.S. beef cattle and four haplotypes were identified (1 through 4), with allele frequencies of 0.51, 0.34, 0.14, and 0.01, respectively. Haplotype 1 was present in all 17 breeds tested, whereas haplotype 4 was only present in the Brahman and Beefmaster breeds tested. We plan to perform a series of experiments designed to determine whether these haplotypes are associated with increased/decreased NOS2 activity and ultimately bacterial killing. Results of these studies may provide an additional tool for producers to use in the selection of cattle superior in the control of pathogenic bacteria.

HOST-MICROBE INTERACTIONS (207-215)

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Uroepithelial cells require TLR4, CD14 and proteases for induction of proinflammatory responses when exposed to LPS

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We recently showed that mucosal recognition of *Escherichia coli*, the most common causative agent of urinary tract infections, correlates with expression of the LPS receptor Toll-like receptor (TLR) 4. It was suggested that the rapid production of proinflammatory cytokines from a bladder epithelial cell line exposed to LPS was dependent on the TLR4 signaling pathway. Here we present a detailed mechanistic analysis of this signaling event. Overexpression of a truncated form of TLR4 in T24 cells abolish the LPS-induced production of IL-6 and IL-8. This demonstrates that epithelial cells also require TLR4 for LPS-stimulated cytokine production as previously has been reported for macrophages. In addition, myeloid cells require the presence of the coreceptor CD14 for efficient LPS signaling. We present data to show that bladder epithelial cells are dependent on CD14 for efficient LPS signaling although these cells do not express endogenous CD14. Thus, the LPS-responsive phenotype of bladder epithelial cells is dependent on soluble CD14 present within body fluids. Activation of the Toll signaling pathway in *Drosophila* involves an upstream proteolytic cascade generating an endogenous peptide which acts as a ligand to the Toll receptor. When investigating the involvement of upstream protease activity in mammalian TLR activation, we screened

a variety of protease inhibitors for their ability to block LPS-stimulated IL-8 release from T24 cells. The ones that inhibited the TLR signaling pathway also abrogated the IL-1R signaling pathway, suggesting that these inhibitors act downstream of TLR4. Detailed analysis of the target(s) of these protease inhibitors showed that they prevent I- κ B degradation, thus prohibiting NF- κ B translocation to the nucleus. Currently, we have no evidence that point to the existence of a LPS-specific protease inhibitor.

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Differential Bactericidal Functions Stimulated by the Activation of Toll-Like Receptors in Chicken Heterophils

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The innate immune response protects against extracellular pathogens and utilizes pattern recognition receptors (PPRs), which in turn recognize molecularly conserved components known as pathogen-associated molecular patterns (PAMPs). Heterophils are essential in establishing an innate immune response in neonatal chickens; however, the functional activity and the mechanism(s) of action involved are not fully understood. The ability of either *Salmonella enteritidis* (SE) and lipopolysaccharide (LPS) or *Staphylococcus aureus* (SA) and lipoteichoic acid (LTA) to promote degranulation and oxidative burst in heterophils isolated from neonatal chicks was evaluated. Immune IgG opsonized SE was used as a positive control, which stimulates both activities through the Fc receptor. Treatment of heterophils with SE, LPS, SA, and LTA decreased degranulation activity by 76%, 78%, 90%, and 90%, respectively from the opsonized SE control. In addition, oxidative burst was evaluated using a quantitative luminol-dependent chemiluminescence assay. SE, LPS, SA, and LTA all stimulated a significant oxidative burst in heterophils that was dramatically reduced by the addition of polyclonal antibody to Toll-like receptors 2 and 4. Therefore, Toll-like receptors mediate oxidative burst but not degranulation, indicating at least two distinct pathways are involved in the function of heterophil killing.

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Regulation of proinflammatory cytokines in human lung epithelial cells by *Mycoplasma pneumoniae* infection

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Mycoplasma pneumoniae is a human pathogen that causes tracheobronchitis and atypical pneumonia. Previous studies have shown that it can induce proinflammatory cytokines in several different systems including mice and/or human monocytes. In this study we demonstrated that *M. pneumoniae* infection also induced proinflammatory cytokine expression in human lung epithelial cells. The mRNA levels for IL-1 β , IL-8, and TNF- α were increased after *M. pneumoniae* infection, while the mRNA levels for IL-6 and IFN- γ remained unchanged. Furthermore, IL-8 and TNF- α proteins were secreted into the culture medium. In contrast, while IL-1 β protein was synthesized, it remained intracellular. Using protease digestion and antibody blocking method, we found that *M. pneumoniae* cytoadherence is important for the cytokine induction. On the other hand, *M. pneumoniae* protein synthesis and DNA synthesis do not appear to be prerequisites for the induction. Also, *de novo* protein synthesis in the lung epithelial cells is responsible for the increased cytokine protein levels. These results suggest a novel role for lung epithelial cells in the immune response to *M. pneumoniae* infection and provide a better understanding of *M. pneumoniae* pathogenesis.

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The role of galectin-3 in resistance against tumor metastasis and infection. Abdul Ghaffar (1), Maurice Nachtigal (2) and Eugene P. Mayer (1). (1)Microbiology and Immunology and (2)Pathology, USC School of Medicine, Columbia, SC

Galactin-3 (Gal3) is a β -galactoside binding protein expressed in many normal tissues including monocytes, macrophages and PMN and its concentration increases as these phagocytes mature. Macrophages are involved in surveillance against malignancy and infections and when activated, they are very effective in killing tumor cells and pathogens. Since phagocytes are involved in protection against both tumor and infection, we examined the role Gal3 in this protection. In one set of experiments, Gal3 knockout (Gal3KO) and wild-type (129/J and C57Bl/6) mice were injected intravenously with B-16 melanoma and 13 days later, examined for tumor foci in lungs, liver, lymph nodes, and spleen. In C57Bl mice, tumor foci were found only in the lungs and no foci were detectable in 129 mice. By contrast, tumor foci were found in all these organs of Gal3KO mice. In addition tumor foci in the lungs of C57Bl mice were fewer in number and smaller than those seen in Gal3KO mice. In another set of experiments, Gal3KO and 129/J mice were challenged, intraperitoneally, with 1x10⁵ *Salmonella enteritidis* (previously known as *S. typhimurium*) and observed daily for mortality. In these experiments, Gal3KO mice had a higher mortality and had shorter mean survival time than their wild-type 129J cohorts. These data indicate that Gal3 may play a role in resistance to both malignancy and infection. It is, however, not certain that this susceptibility is due to ineffective phagocyte functions.

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Activation of Innate Immune Responses by Intracellular Pathogens

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The innate immune responses to two intracellular pathogens were compared. *Rhodococcus equi* is a Gram-positive bacterium which can cause pneumonia in immunocompromised humans. *Leishmania* spp. are protozoan parasites which can cause cutaneous leishmaniasis in immunocompetent individuals. Both of these pathogens reside primarily, if not exclusively, within host tissue macrophages. We examined macrophage cytokine responses to infection with *R. equi* and *Leishmania* promastigotes. Measurement of IL-12 p40 and TNF α revealed that macrophages produce only minimal amounts of either cytokine following *Leishmania* infection. In contrast, infection with *R. equi* elicited the brisk production of both IL-12 p40 and TNF α transcript and protein. Additionally, rapid nuclear translocation of NF- κ B, a transcription factor of particular importance in regulating the IL-12, TNF α , and iNOS genes was observed following bacterial but not parasitic infection of macrophages. The activation of NF- κ B and the induction of macrophage cytokine gene transcription by *R. equi* correlated with their interaction with Toll-like receptor type 2 (TLR2) on macrophages. *Leishmania*, in contrast, failed to interact with TLR2 and failed to translocate NF- κ B in infected macrophages. This resulted in a lack of TNF α production and a failure to induce nitric oxide production. Coinfection of macrophages with *Leishmania* and *R. equi* resulted in a suppressed level of *R. equi*-induced IL-12 p40. This *Leishmania*-mediated suppression was specific to IL-12 p40 and was not due to an inhibition of NF- κ B translocation. Thus, we demonstrate that two different intracellular pathogens elicit divergent responses from the macrophages that they infect. The bacterium, *R. equi*, evokes a vigorous proinflammatory response which effectively resolves infection but also mediates significant tissue damage to the host. In contrast, *Leishmania*, evades macrophage proinflammatory responses. This accounts for the delayed onset of cell-mediated immune responses and may contribute to the success of *Leishmania* as a pathogen.

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The response of Tlr4-receptor-positive cells to pulmonary infection.

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Toll-like receptor 4 (Tlr4) is a trans-membrane receptor that is the primary lipopolysaccharide (LPS) recognition molecule of the macrophage for Gram-negative bacteria. The *Tlr4*^{Δd} mouse strains C57BL10/ScN and BL10/ScN x C2D have an increased susceptibility to pulmonary infections caused by *Pasteurella pneumotropica*. From these data we hypothesized that Tlr4 is a necessary receptor for early recognition and response to Gram-negative bacteria-induced pulmonary infection. To test this hypothesis, we studied C57BL10/ScN *Tlr4*^{Δd} mice that were reconstituted with whole bone marrow from syngeneic *Tlr4*

reported in Ld-infected MØs, however, the mechanisms involved are still unclear. To address this question, IFNγR expression was measured by FACS analysis on human elutriated monocytes M-CSF derived MØs, and found to be rapidly decreased after infection. Decreased IFNγR expression after Ld infection corresponded to a direct induction of Suppressor Of Cytokine Signaling-3 (SOCS-3) mRNA, a molecule involved in specific deactivation of IFNγ signaling, while other SOCS family proteins were not transcribed. SOCS-3 has been proposed (1) to inhibit Jak/STAT activation and signal transduction and (2) to target receptor chains to the proteasome for degradation. SOCS-3 may play such a role in Ld-infected MØs, accounting for both reduced Jak/STAT phosphorylation and IFNγR expression. Together, these results suggest for the first time that, infection of MØs by Ld results in a direct suppressive effect on MØ activation via induction of SOCS-3 and inhibition of IFNγ signaling.

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MEK/ERK-dependent Signaling and the NADPH Oxidase are Essential for Inhibition of *Salmonella enterica* serovar Typhimurium Replication by Murine RAW 264.7 Macrophages

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Host responses during the later stages of *Salmonella*-macrophage interactions are critical to controlling infection but have not been well characterized. After 24 h of infection, nearly half of IFN-γ primed murine RAW 264.7 macrophage-like cells infected by *Salmonella enterica* serovar Typhimurium contained filamentous bacteria. Bacterial filamentation indicates a defect in completing replication and has been previously observed in bacteria responding to a variety of stresses. To understand whether macrophage gene expression was responsible for this effect on *S. Typhimurium* replication, we used gene arrays to profile IFN-γ primed RAW 264.7 cell gene expression following infection by *S. Typhimurium* for 8 h and 24 h. We observed an increase in MEK1 kinase mRNA at 8 h post-infection with a corresponding increase in MEK protein and kinase activity detectable at 24 h. Treatment of cells with MEK kinase inhibitors significantly reduced numbers of filamentous bacteria observed within macrophages after 24 h and increased the number of intracellular CFU. This supports a role for MEK/ERK signaling in mediating a host bacteriostatic pathway. MEK/ERK signaling can activate both the NADPH oxidase and iNOS and NADPH oxidase inhibitors and antioxidants significantly reduced bacterial filamentation while iNOS inhibitors had no significant effect on bacterial morphology. In summary, *S. Typhimurium* infection of IFN-γ primed macrophages triggers a MEK kinase cascade at later infection times and both MEK kinase and NADPH oxidase impair bacterial replication. Signal transduction by MEK kinase and NADPH oxidase may play an important role in innate host defense against intracellular pathogens.

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An impaired interferon gamma signaling pathway in Leishmania-infected human macrophages is associated with the induction of Suppressor Of Cytokine Signaling-3

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The parasite *Leishmania donovani* (Ld) is the causative agent of lethal human visceral leishmaniasis. Survival of Ld within the host macrophage (MØ) is attributed to parasite-mediated inhibition of MØ activation and correlated in part to alteration of the interferon gamma (IFNγ) signaling pathway. IFNγ signals through its receptor (IFNγR) by involving transient increase in the activities of cellular protein tyrosine kinases Jak1 and Jak2 leading to tyrosine phosphorylation of the transcription factor STAT1. Phosphorylated STAT1 dimerizes and translocates into the nucleus to activate gene transcription. A reduced phosphorylation of Jak1, Jak2 and STAT1, along with increased protein tyrosine phosphatase activity and reduced IFNγR expression have been

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Leishmania species differentially modulates macrophage migration and expression of cell adhesion molecules

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Leishmaniasis is a vector-borne parasitic disease which, depending mainly upon the species of *Leishmania*, can display a spectrum of clinical manifestation ranging from localized cutaneous lesions that heal spontaneously to generalized systemic disease with fatal outcome. Regardless of species, all *Leishmania* infections are initiated by sand fly derived metacyclic promastigotes which are deposited in the skin where they are rapidly taken up by macrophages and replicate as intracellular amastigotes. Factors that favor the dissemination of certain parasite species (e.g. *L.donovani*) from the skin to internal organs have not been identified. We have developed a system to compare the ability of macrophages infected with different species of *Leishmania* to migrate out of the dermal matrix. Murine macrophages were infected with different strains of *L. major* or *L. donovani*, labeled with a fluorescent dye and injected in the ears of C57Bl/6 mice. Twenty four hours later, the ears were recovered, and the cells sedimenting from the dermis during 16 h were collected and analyzed by FACS. Macrophages infected with visceralizing species migrated out of the dermis in significantly greater numbers compared with cutaneous species. Infected cells could be also tracked in the draining nodes. The differences in migratory behavior correlated with the expression of cell adhesion (CD62L, CD11b, CD49, CD54) and co-stimulatory molecules present on the surface of infected cells. These results suggest that dissemination of *Leishmania* is a direct consequence of species restricted, parasite driven changes in infected host cells that affect their adhesion and migratory properties.

HOST-MYCOBACTERIAL INTERACTIONS (216-220)

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Expression of Iron Transport Protein mRNA in Murine Macrophages were differentially Regulated by *Mycobacterium avium* Infection.

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The iron transporter, natural resistance associated macrophage protein 1 (Nramp1) confers resistance of macrophages to the growth of intracellular bacteria, such as *Mycobacterium avium*. A highly homologous iron transporter Nramp2 has also been described. Transferrin receptor (TfR) and hereditary hemochromatosis (HFE) protein are also associated cell surface proteins controlling iron import. The relationship of these proteins to host defense and the growth of intracellular pathogens are not clear. Here, we report the differential expression of these iron transporters in thioglycollate-elicited mouse peritoneal macrophages following infection by *M. avium*. Nramp2

mRNA reached a peak after 4 to 8 hours and remained elevated up to 48 hours. Nramp1 mRNA increased slowly and reached a peak at 20 hours after infection. TfR mRNA began to decrease after 8 hours of infection and declined maximally by 20 hours after infection. The level of HFE mRNA remained constant. Treatment of macrophages with the inflammatory mediators LPS or TNF- α increased the mRNA levels of Nramp1 and Nramp2 but decreased that of TfR. These results suggest that the differential expression of these iron transporters is important in regulating availability of iron during infection with an intracellular pathogen. (This work is supported by grants DK-57667, AI-42901 and HL-59795 from National Institutes of Health to B.S.Z. and W.P.L.)

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***Mycobacterium avium* Regulation of Macrophage Cytokine Production**
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In immunocompromised individuals, the otherwise avirulent *M. avium* becomes an opportunistic pathogen and colonizes the host. To decipher the mechanisms by which *M. avium* interacts with its macrophage hosts in the absence of T cells, viable mycobacteria were exposed to adherent macrophages for 5 min to 2 hrs and signal transduction, gene expression and protein levels monitored. The initial macrophage-mycobacteria interaction was largely TLR2 dependent, blocked by TLR2 antibody, and mimicked by exposing cells to purified *M. avium* lipoarabinomannan, a TLR2 ligand. Phosphorylation of MAP kinase p38 within minutes resulted in downstream signal transduction, including NF κ B activation, transcription and synthesis of inflammatory and regulatory proteins. Among the NF κ B-inducible proinflammatory genes were chemokines, adhesion molecules, TNF α and IL1 β . The IL1 β and TNF α genes were upregulated within 2 hours by *M. avium*. TNF α and IL1 β protein by ELISA revealed high levels of TNF α , but no IL1 β . The dichotomy between IL1 β mRNA and protein suggested a post-transcriptional block and/or a potential failure to cleave proIL1 β to IL1 β . IL1 β convertase gene expression, but not protein, was transiently enhanced by *M. avium*. By suppressing proteolysis of the IL1 β precursor into its biologically active form, *M. avium* may enable its own growth within intracellular vacuoles in macrophage cultures lacking T cells and/or in the absence of T cell activating factors in immunocompromised hosts. The absence of IL1 β signaling via the toll/interleukin 1 receptor signal pathway and its antipathogen response may favor *M. avium* survival.

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Roles of free fatty acids in expression of the antimicrobial activity of macrophages against *Mycobacterium tuberculosis*

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We previously found that free fatty acids (FFA), including arachidonic acid (AA), play important roles in expression of antimycobacterial activity by macrophages (M ϕ s). In this study, we examined the role of AA in expression of M ϕ anti-*M. tuberculosis* (MTB) activity, in relation to the roles of reactive nitrogen intermediates (RNI) and reactive oxygen intermediates (ROI). First, intramacrophage growth of MTB was accelerated by treatments of M ϕ s with either quinacrine (phospholipase A2 [PLA2] inhibitor), a-TFMK (type IV cPLA2 inhibitor), NMMA (NOS inhibitor), or SOD plus catalase (ROI scavengers). In addition, MTB-infected M ϕ s produced and/or secreted these effectors sequentially in the order ROI, AA, and RNI. Second, membranous AA of M ϕ s translocated to MTB residing in M ϕ phagosomes in phagocytic ability- and Type IV cPLA2-dependent fashions during cultivation after *M. tuberculosis* infection. This AA translocation was not affected by NDGA (lipoxygenase inhibitor) and indomethacin (cyclooxygenase inhibitor), indicating that AA molecules themselves translocated to intramacrophage MTB. In addition, IFN- γ up-regulated M ϕ anti-MTB activity in parallel with the increase in the mRNA expression of Type IV cPLA2 but not of Type IIa sPLA2. Third, AA, RNI, and H2O2-halogenation system displayed strong activity against MTB, while ROI alone exerted no such effects. Combinations of α -AA+RNI and α -RNI+H2O2-halogenation system exhibited combined

effects against MTB, whereas α -AA+H2O2-halogenation system had an antagonistic effect. Moreover, a sequential attack of AA followed by RNI exerted synergistic activity against M ϕ , indicating important roles of RNI and FFA in M ϕ anti-MTB activity.

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Cell-to-cell contact-dependent expression of the suppressor activity of *Mycobacterium avium* complex-induced immunosuppressive macrophages against target T cells

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Immunosuppressive M ϕ s induced by *Mycobacterium avium* complex infection (MAC-M ϕ s) transmit suppressor signals to T cells not only by producing immunosuppressive mediators, but also by directly binding to target T cells. We have examined the mode of cell contact between MAC-M ϕ s and target T cells, particularly what kinds of M ϕ surface molecules are required for such cell-to-cell interaction. First, the suppressor activity of MAC-M ϕ s was reduced by paraformaldehyde-, cytochalasin B-, or colchicine-treatment, indicating that vital membrane functions are required for functional cell-to-cell contact. Second, blocking experiments using mAbs against several adhesion molecules indicated that a B7-1-like molecule (B7-1MAC-M ϕ), which was recognizable with only one anti-B7-1 mAb of peculiar clone among the test mAb clones, was required for expression of the suppressor activity of MAC-M ϕ s. Moreover, MAC-M ϕ s displayed increased B7-1MAC-M ϕ expression that was parallel with their suppressor activity and bound to T cells in a B7-1MAC-M ϕ -dependent manner. Notably, the Ab blocking of CD28 and CTLA-4 on target T cells did not reduce the suppressor activity of MAC-M ϕ s. Therefore, on target T cells, there may exist some novel molecule other than CD28 and CTLA-4, that functions as the receptor for B7-1MAC-M ϕ and acts as a negative regulator of T cell activation. In addition, Con A-induced stimulation of MAC-M ϕ s, that is needed for effective expression of their suppressor activity, was inhibited by KN-62 but not by herbimycin A, H-7, nor H-88. This indicates important roles of calmodulin-dependent protein kinase II and/or P2X7 purinoceptors in expression of the suppressor action by MAC-M ϕ s.

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A Single Vaccination With Protein-Microspheres Elicits a Strong CD8 T-Cell Mediated Immune Response Against *Mycobacterium tuberculosis* Antigen Mtb8.4

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Mycobacterium tuberculosis (Mtb) is a leading cause of infectious mortality worldwide with > 8 million new cases and 2.9 million deaths annually. The only available vaccine against Mtb is the *M. bovis* bacillus Calmette-Guérin (BCG) vaccine that was developed nearly a century ago and results in highly variable protection. Significant progress has been made towards the identification of novel Mtb antigens. However, an efficient delivery system is needed to achieve a persistent memory immune response capable of detecting and eliminating an intracellular pathogen such as Mtb. We have developed a novel protein-microsphere based delivery system using the newly discovered Mtb antigen Mtb8.4. Microsphere formulations with varying *in vitro* release kinetics were tested for *in vivo* responses to antigen following subcutaneous administration. The strongest cell mediated and antibody responses were obtained using microsphere formulations that exhibited slow, controlled release *in vitro* over the course of several weeks. Immunization of mice with a single dose of Mtb8.4-microspheres resulted in a strong CD8 T-cell mediated immune response. In addition, immunized mice produced high levels of Mtb8.4 specific IgG₁ and IgG_{2b} antibodies. The percentage of Mtb8.4 specific CD8⁺IFN γ ⁺ T-cells following a single administration of Mtb8.4-microspheres was similar to that observed following three protein plus adjuvant or plasmid DNA-immunizations. These results demonstrate the efficacy of a single dose protein-microsphere vaccine for the induction of strong cell-mediated and antibody responses against Mtb antigens.

INFECTIOUS DISEASES (221-226)

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A Limulus anti-LPS factor (LALF)-derived peptide modulates cytokine gene expression and promotes resolution of bacterial acute infection in mice.

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Sepsis in human and experimental animals has been associated with a perturbed immune response. A major event contributing to the decrease in immune functions in septic disorders seems to be the inadequate balance of cytokines mediating the interaction between the innate and adaptive immune system. We herein examined the effects of the LALF31-52 peptide in an experimental model of Gram-negative peritoneal sepsis and analyzed the cytokine gene expression in spleen and liver of peptide-treated mice. Histological examination of spleen and liver in peptide-treated mice showed prevention of tissue damage induced by the high dose of *P.aeruginosa*. This treatment modulates cytokine gene expression in these tissues, stimulating IL-2, IL-12 and IL-13 synthesis, while IL-4 and IL-10 mRNA expression was not modified. This cytokine profile induced by the LALF peptide seems to be favorable for host resistance against Gram-negative bacteria acute infection. These results further demonstrate the immunomodulatory potential of LALF31-52 and are relevant for the design of prophylactic and therapeutic strategies for acute bacteria infection and sepsis, specially for preventing or ameliorating host immunity defects in these disorders.

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IL-12 increase during neutropenia in established infections

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The pivotal role of IL-12 in the humoral and cellular immune systems has made this cytokine important to the host immune defense against infection. Staphylococcal superantigens are known to induce both IFN γ and IL-12(p40) production. Understanding the interactions between pathogens and IL-12 would significantly benefit the treatment of infectious disease. To that end, long-term, encapsulated abdominal infections were established in a rat model with *Staphylococcus aureus*, n=17. Nine rats were made neutropenic (anti-rat neutrophil antibody, administered on days -1, 2, 4, and 6). Infection exudate was sampled aseptically through the abdominal wall at 0.5, 2 and 4 weeks of infection. Differential cell counts and bacterial counts by dilution plating were performed on all samples. Infection exudates were sterile-filtered and stored at -70°C until assay. An ELISA for rat IL-12 (both p35 and p40) was run using manufacturer's directions. IL-12 was significantly increased in neutropenic infection exudate, p=0.01. On day 3 of infection, neutropenic rats had 1190 \pm 1926 pg/mL IL-12, and the control infected had 341 \pm 682. By day 14, neutropenic rats had 1941 and controls had 152 pg/mL. The *S. aureus* count, higher in the control group exudate than in the neutropenic animals, suggests that staphylococcal superantigen stimulation does not explain the significant jump in IL-12 in the neutropenic animals. Ionized calcium, reported to be important in neutrophil-IL-12 interaction, was measured in all exudates, and there was no significant difference in this ion between the groups. IL-12 receptors have been identified on neutrophils, and based on neutrophil numbers during infection, these cells would constitute a major receptor site for IL-12. The present data are consistent with the proposal that IL-12 production increases during infection when neutrophil IL-12 receptors are unavailable. Experiments continue to clarify these issues.

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The immune response during persistent Salmonella infection

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Using *Salmonella typhimurium* in a murine model, we are investigating the immunologic basis underlying persistent *Salmonella* infection. A single oral

dose of a virulent *S. typhimurium* strain causes a persistent infection in 30-50% of surviving mice. Persistent infection is characterized by splenomegaly and bacteria (100-10000) recovered from spleen and liver 7-27 weeks after inoculation. Mice with persistent infection have high serum titers of *Salmonella*-reactive IgG2a, IgG1, IgM and IgA. This serum contained high reactivity against lipopolysaccharide and recognized several *Salmonella* proteins. The total number of cells in the spleen and the liver of persistently infected mice was 2-6 times greater than the number of total cells in cleared mice (mice that received bacteria but did not become persistently infected). FACS analysis of splenocytes showed no changes in the T cell compartment or in the number of B220+ cells of persistently infected mice while increased numbers of CD11c+MHC-II+ and Gr-1+ cells were apparent compared to cleared mice. FACS analysis of liver cells showed a slight increase in the number of T cells, CD11c+MHC-II+ and B220+ cells and a major increase in Gr-1+ cells in the persistently infected mice compared to cleared mice. IFN- γ and TNF- α was detected by immunohistochemistry in the red pulp of the spleen of persistently infected mice. Furthermore, IFN- γ was produced by *Salmonella*-specific CD4+ and CD8+ cells from both spleen and liver of persistently infected mice detected by FACS analysis.

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Immune Mechanisms of Protection from Rotavirus Challenge

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Rotavirus is the leading cause of acute diarrheal disease in children worldwide. However, the immunological mechanisms of protection from rotavirus infection remain to be defined. Based on analyses of antibody isotypes and IgG subclasses in serum and fecal samples following rotavirus infection, we hypothesize that immunity following rotavirus infection is mediated by intestinal IgA through TH1-like and TGF- β cytokine responses. We propose the involvement of TGF- β in rotavirus protection based on several observations. First, there are large IgA titers following rotavirus infection, despite the lack of hallmarks of a TH2 cytokine response. Second, a dominant IgG2b response is seen in mouse intestine. Third, there is limited inflammation of the intestine during rotavirus infection. Using RNase protection assay, we found TGF- β 1 mRNA was upregulated as early as 1 days post infection (dpi) in the spleen and continued to be upregulated in the spleen, MLN, and PP of rotavirus-infected mice until at least 8 dpi. Total TGF- β 1 protein levels were increased in the sera of rotavirus-infected mice from 1 to 7 dpi and again from 14 through 21 dpi. The source of this TGF- β is unknown, but TGF- β is produced by many cell types, including epithelial cells, which are the primary target of rotavirus infection in vivo. We are currently examining the effect of rotavirus infection on TGF- β 1 levels in HT-29 cells, a human intestinal epithelial cell line. Rotavirus infection appears to upregulate TGF- β 1, which likely mediates regulation of inflammation, intestinal healing, and control of the immune response. Little is known about the mechanisms of immune regulation to virus infections of the intestine, our studies are beginning to define the basic mechanisms in response to rotavirus.

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CYTOKINE PREDICTIVE VALUES OF BACTEREMIA AND MORTALITY IN THE ELDERLY WITH FEVER

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Background: Proinflammatory cytokines (IL-1b, IL-6, TNF-a) are excellent predictors for tissue damage, inflammation and infection. Objective: To define which cytokines are related to bacteremia and which ones are predictors for bad prognosis in the elderly patient with fever. Methods: Prospective study performed in 1999. Patients aged \geq 60 and T \leq 38°C admitted to the ED. Parameters required: IL-1b, IL-6, TNF-a, CRP and cultures. On the 4th day, cytokines, CRP and evolution were recorded. Results: 100 patients were included. Median age: 75 years (SD 8.4). APACHE II score at ER: 16.6 (SD: 4.7). In 43 (43%) the etiology showed some microorganism. 28

patients presented bacteremia: E.coli(13), S. pneumoniae(5), Staph coagulase negative(3) and others in 7. 14 patients died (14%). Median values at ER: CRP: 7.6 mg/dl (N < 0.8), TNF- α : 35.5 pg/ml (N < 20), IL-1b: 2 pg/ml (N < 15) and IL-6: 130.5 pg/ml (N < 5). Values on the 4th day were 5.3 mg/dl, 32.5, 1 and 45 pg/ml respectively. Initial TNF- α and the 2nd values were significantly superior in the patients with bacteremia, as well as the initial IL-6 values $p < 0.05$. The follow-up values of IL-6 were superior in patients who died ($p < 0.001$). Conclusions: All median values of TNF- α and the 2nd values of IL-6 were higher in the studied population with bacteremia. Patients who died had higher IL-6 levels in their follow-up. IL-1b was significantly higher only in the first determination in respiratory versus urinary sepsis. CRP was not predictive for patients with bacteremia neither for those who died.

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Inhibition of IL-12 production by hepatitis C viral proteins correlates with reduced APC function of dendritic cells from HCV infected patients

Angela Dolganiuc, Karen Kodys, Andrea Kopasz, Pranoti Mandrekar, Gyongyi Szabo. University of Massachusetts Medical School, Worcester, MA. Hepatitis C, the most common cause of hepatitis in the USA, results in chronic infection due to insufficient viral clearance indicating consistent defects of the immune system in viral recognition and elimination. Viral recognition, at least partly, is dependent on antigen-presenting dendritic cells (DC). Here we investigated the hypothesis that monocyte-derived DC accessory cell function is reduced in patients with chronic HCV infection and that this effect is mediated via HCV core or NS3 proteins. Myeloid DCs were generated from adherent blood monocytes in the presence of IL-4 (0.1 μ g/ml) and GM-CSF (0.01 μ g/ml) for 7 days from normal controls and from 30 HCV positive, untreated patients. The morphologic appearance and the expression of DC phenotypic markers (HLA-ABC, HLA-DR, CD80, CD83, CD86, CD1a, CD40, DC-LAMP) were not different between DCs from HCV patients and controls. In contrast, we found significantly reduced allostimulatory capacity of DCs from HCV patients compared to normal DCs ($p < 0.01$) in a one-way mixed lymphocyte reaction. Reduced APC function correlated with a significant decrease in IL-12, IL-1Ra, IL-6, and IFN- γ mRNA levels in DCs of HCV patients compared with control DCs in RPA. Further, the increased levels of IL-10 mRNA seen in DCs of HCV patients may contribute to the reduced accessory cell function of HCV patients' DCs. Cultivation of normal DCs for 7 days in the presence of 10 μ g/ml of recombinant core or NS3 HCV protein significantly reduced ($p < 0.05$) accessory cell function in MLR as well as decreased IL1Ra, IL-6, IL-12, MCP-1 and IFN- γ secretion, while IL-8 production was increased compared with control DCs. Both IL-12 and IFN- γ secretion were reduced during MLR in the presence of dendritic cells exposed to HCV in vivo or to HCV proteins in vitro. There was no increase in apoptosis of DCs from HCV patients or in cells generated with HCV proteins comparative with controls. In vitro IFN- α administration partially restored reduced IL-12 secretion in HCV protein-treated DCs suggesting a possible mechanism of this antiviral agent in HCV treatment. Our data support the hypothesis that impaired DC accessory cell functions contribute to immune abnormalities in chronic hepatitis C infection, and are likely mediated by direct effects of HCV viral proteins on dendritic cell maturation/functional capacity.

AIDS (227-229)

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CCR5 variants permissive for HIV-1 Infection Show Distinct Functional Responses to CCL3, CCL4 and CCL5

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Human CCR5 is the primary co-receptor for Env-mediated fusion by human immunodeficiency virus type 1 (HIV-1). Analyses of CCR5 variants in HIV-1 high risk, cohorts lead to the identification of multiple single amino acid

substitutions in the co-receptor CCR5, suggesting the possibility of protective and permissive HIV-1 genotypes. This study focused on eight naturally occurring allelic variants located between amino acid residues 60 and 334 of CCR5 and we investigated the ability of these variants to support HIV-1 infection, the effect of these variations on chemokine binding, cell migration and calcium mobilization. All allelic variants studied were highly expressed on the cell surface and permissive for HIV-1 infection. Four variants, S215L, R223Q, G301V, R334Q, resulted in moderately increased CCL5 (RANTES) binding affinity which did not affect CCL5-induced chemotaxis. The G301V variant showed decreased CCL4 (MIP 1 beta) binding affinity, while all other C-terminal variants had no effect. The R60S mutant showed a decreased CCL4 binding affinity, but no effect on CCL5 binding affinity. Interestingly, this R60S mutant did not respond to CCL5 with the classical bell-shaped chemotactic response curve, but rather yielded a saturation curve and exhibited a loss of CCL5-induced desensitization. This mutation is located in the first intracellular loop, a domain that has not previously been shown to be involved in receptor desensitization. In conclusion, this study demonstrated that single amino acid changes between 60 and 334 residues resulted in a variety of effects on ligand-induced functions without affecting the susceptibility of individuals to HIV, but may have immunological consequences.

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Multiplexed Cytokine Assay of Serum from HIV-Infected Patients with Differing Disease Presentations

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Advances in technology for measuring a number of cytokines in a multiplexed fashion from single, small (25 μ l) fluid samples has permitted more intensive analyses of cytokine induction in patients. Levels of 8 cytokines (IL-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, IFN- γ , and TNF- α) were measured simultaneously in the serum of HIV-infected patients. These individuals were chosen to represent a number of different disease presentations associated with HIV infection. Disease presentations included Kaposi's Sarcoma (KS, n=12), non-Hodgkin's AIDS Lymphoma (NHL, n=12), Progressive Multifocal Leukoencephalopathy (PML, n=5) and Wasting Syndrome (WAS, n=5). In addition, patients with Classical Kaposi's Sarcoma (HIV-negative) were assessed (CKS, n=4). Analysis was performed using beads with graded fluorescence conjugated to specific anti-cytokine antibodies (LINCOplex, Linco Research), measured on a Luminex 100 cytometer. In general, differences in serum cytokine levels were small. Highest levels of most cytokines were seen in the NHL group, and lowest levels were seen in the WAS patients. For many cytokines (IL-2, IL-4, IL-6, IL-1 β), levels were higher in infected patients with KS, NHL and PML, compared to CKS patients. No distinct patterns of serum cytokine induction were associated with any specific disease state.

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Elevated Inflammation-Related Transcripts in HIV-Infected Individuals are Decreased after Administration of 16- α -Bromoepiandrosterone (HE2000) - an immunostimulatory steroid

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Gene expression was analyzed in HIV-infected individuals before and after HE2000 treatment. PBMC were prepared from 7 healthy South African donors and 41 patients enrolled in studies of subcutaneous (CD4 > 200) or intramuscular (CD4 < 100) HE2000 administration before and after 4 days of treatment with drug or vehicle, performed by real-time RT-LightCycler PCR. Levels of transcripts relating to immune regulation were normalized to transcripts of 2 housekeeping genes. HIV patients (CD4 > 200) pretreatment demonstrated significant ($p < 0.001$) increases over healthy controls for IL1 β , TNF α , MIP1 α , IFN γ , IL6, IL8, IL10 and COX2. HE2000 decreased IL1 β (-30% $p=0.007$), IL6 (-45% $p < 0.001$), IL8 (-22% $p=0.005$) and COX2 (-48%

$p=0.04$), and increased PPAR γ (+49% $p=0.005$). HIV patients (CD4 < 100) showed decreases from baseline values compared to vehicle of -260% for IL1 β , -35% for TNF α , -610% for IL6, -147% for IL8, -86% for COX2, and -18% for GATA3, and increases of 118% for IFN α and 85% for Tbet. Control injections increased inflammation; HE2000 decreased inflammation. We conclude that HE2000 administration in HIV-1 infected patients significantly decreases gene transcripts for pro-inflammatory cytokines and enzymes and increases expression of genes associated with anti-inflammatory nuclear receptors and improved cellular immunity. HE2000 treatment may decrease immunosuppression and progression to AIDS.

VIRAL-CYTOKINE MECHANISMS (230-234)

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VIRAL INFECTION INDUCES TOLL-LIKE RECEPTOR GENE EXPRESSION

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Toll-like receptors (TLRs) have been shown to mediate innate immune responses to microbes. Microbial engagement of TLRs leads to transcription factor activation, cytokine production, enhanced MHC expression, and nucleosome remodeling. TLR2, TLR4, TLR5, and TLR9 are involved in responses to bacteria. Recently, TLR4 has been implicated also in responses to respiratory syncytial virus. We have analyzed the expression of TLR1-9 in human primary macrophages as well as in epithelial and endothelial cells during influenza A and Sendai virus infection. Our results show that in macrophages TLR1, TLR2, TLR3, and TLR7 mRNA expression was induced by both viruses. Virus-induced TLR3 and TLR7 expression was inhibited by neutralizing antibodies to interferon- α (IFN- α). In addition, purified IFN- α up-regulated TLR1, TLR2, TLR3, and TLR7 gene expression in macrophages. In epithelial and endothelial cells TLR3 mRNA was induced by viruses, IFN- α and IFN- γ . The data suggests a novel role for IFNs in the activation of innate immunity.

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Variations in serum IL-7 and 90K/MAC-2 binding protein levels analysed in cohorts of HIV-1 patients and correlated with clinical changes following antiretroviral therapy.

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Serum levels of IL-7, a non-redundant cytokine that plays a crucial role in lymphopoiesis, are known to be elevated in HIV-1-infected subjects. To further examine the association between levels of IL-7, CD4 cell counts, and viraemia, we analysed these parameters in a large cohort of HIV-1 patients along with serum levels of 90K, a marker of disease severity but with no established involvement in lymphopoiesis. While IL-7 levels were only found to correlate with CD4 counts, 90K levels presented strong correlations with both CD4 cell numbers and with plasma viral loads (VL). These correlations were maintained in patients naive to treatment with antiretrovirals ($n=38$) but were abolished when the analysis was restricted to the group receiving highly active antiretroviral therapy (HAART, $n=82$). Moreover, although 90K levels were significantly reduced in patients on HAART, IL-7 levels continued to be elevated despite successful treatment. The influence of HAART on the variations in these serum parameters was further assessed in a longitudinal study on 32 subjects. The HAART-induced decrease in VL and increase in CD4 counts were found to correlate with a reduced serum level of 90K and IL-7, respectively. Nevertheless, following a median period of 33 months of immunologic and virologic successful HAART, serum levels of IL-7 continued to be significantly elevated as compared with those detected in healthy controls. These findings suggest that immunotherapy with IL-7, aimed to replenish T-cell stock in HAART-treated subjects, may have a limited impact on the process of immune reconstitution.

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Oral Interferon Therapy and the Innate/Acquired Axis

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Orally administered type I interferon (IFN α/β) therapy has shown efficacy in various animal models of disease and in human clinical trials. Recent experimental data has suggested that the effects of oral IFN are based on an innate/acquired immune response linkage. Specifically, the type I IFN production during the innate immune response appears to govern both the nature and the extent of the subsequent acquired immune response. We will present data on (distal) white blood cell responses to oral IFN therapy (spleen, lymph nodes and blood). These data indicate extensive cell trafficking and include studies on the modulation of L-selectin over the timecourse of the therapy. A mechanism of action is proposed for oral IFN based on a natural viral defence process in mammals.

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HPV 16 E6 Binds to TRADD and Blocks Activation of the Downstream Apoptotic Pathway

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High risk strains of human papillomavirus (HPV), such as HPV 16, are causative agents in most cases of human cervical cancer. The E6 onco-protein of HPV 16 is best known for its ability to mediate the rapid degradation of the tumor suppressor p53. Evidence has been accumulating, however, that this is not the only function of E6, and indeed, cannot completely explain its transforming potential. We have previously reported that transfection of the human papillomavirus 16 E6 protein into the TNF-sensitive mouse fibroblast LM cells protects expressing cells from TNF-triggered apoptosis in a p53-independent manner. This protection is also evident in human U-2 OS (osteosarcoma) and U937 (histiocytic lymphoma) cells transfected with E6. Differences in the level of NF κ B activation do not account for the differential sensitivity. Also, E6 is not a major inhibitor of the mitochondrial apoptotic pathway, as ceramide and sphingosine induce apoptosis in both LM (E6-negative) and LME6 (E6-expressing) cells in a similar manner. However, caspase 3 activation is blocked in LME6 cells, suggesting that E6 acts upstream of caspase 3 in the caspase-mediated apoptotic pathway. We have found that TRADD (TNF RI-Associated Death Domain), a mediator of TNF-triggered apoptosis, binds to epitope-tagged E6. Such binding could interfere with the ability of TRADD to participate in formation of the Death-Inducing Signaling Complex (DISC) and thus block its ability to transmit apoptotic signals from the surface.

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LIGHT interferes with the HVEM-mediated route of Herpes Simplex-1 infection.

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HVEM, a new member of the TNFR superfamily expressed on T cells and dendritic cells, is a cell surface receptor for LIGHT, LT α , and Herpes Simplex Virus I (HSV) glycoprotein D. The interaction of LIGHT with HVEM induces TRAF recruitment, NF κ B activation, and results in T cell activation. HVEM serves as a route of entry for HSV infection and may be utilized by the virus to target and inactivate cells important for immune responses. LIGHT expression in cells that also express HVEM inhibited HSV infection by reducing surface levels of HVEM. This inhibition occurred when full-length LIGHT was co-expressed with HVEM, whereas a soluble form of LIGHT was much less efficient at inhibiting viral infection. Previous studies showed the membrane LIGHT-HVEM interaction can be blocked by soluble gD, these findings

indicate that the reverse is not true as soluble LIGHT does not block gD-HVEM interaction or HSV-1 infection. HVEM signaling was also modulated by full-length LIGHT: low levels of LIGHT enhanced NF κ B activation whereas the elevated levels that resulted in reduced HVEM surface expression inhibited NF κ B activation. Because HSV infection is dependent on NF κ B activation, these results indicate that LIGHT deters HSV infection by interfering with viral entry through HVEM or by blocking HVEM-dependent NF κ B activation that may be important for viral replication. This work was supported by ACS grant PF0107801LIB and NIH grants AI33068, CA69381, AI48073.

HOST-HIV INTERACTIONS (235-237)

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Double Edged Effect of Vgamma9/Vdelta2 T Lymphocytes on Viral Expression in an in vitro model of HIV-1/M. tuberculosis Coinfection
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 A reciprocal influence exists between mycobacteria and HIV: HIV-infected individuals are more susceptible to mycobacterial infections and, on the other hand, mycobacterial infection results in acceleration of HIV disease progression. Vg9/Vd2 T lymphocytes are known to participate to the defense against intracellular pathogens, including *M. tuberculosis*. Indeed they kill mycobacteria-infected macrophages and, upon recognition of mycobacterial antigens, release TNF- α and IFN- γ which are also upregulators of HIV expression. To assess whether mycobacteria-activated gd T lymphocytes contribute to the enhancement of HIV replication, we established an in vitro model mimicking HIV and *M. tuberculosis* co-infection with the latently HIV-infected promonocytic U1 cell line and Vg9/Vd2 peripheral lymphocytes stimulated with a synthetic mycobacterial antigen. gd T cell activation determined two distinct but connected effects, i. e. U1 cell death and HIV expression. Both effects were mainly mediated by release of TNF- α and IFN- γ from activated gd lymphocytes, although Fas-FasL interaction also contributed to U1 apoptosis. The final outcome on U1 survival, and thus on HIV expression, highly depended on mycobacterial antigen concentration coupled to the differential secretory potency of gd cells. In particular, the induction of viral expression prevailed at low antigen concentration and with lower cytokine production by mycobacteria-activated gd cells. Notably, during the course of HIV infection Vg9/Vd2 lymphocytes are relatively spared and are reported to be functionally impaired. Thus, we suggest that specific recognition of mycobacterial antigens by gd T lymphocytes in co-infected individuals may affect viral replication and indirectly influence the progression of HIV disease.

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INVOLVEMENT OF Bcl-2 AND THE IL-2 RECEPTOR IN HIV+ PATIENTS WHOSE CD4 COUNTS FAIL TO INCREASE SIGNIFICANTLY IN RESPONSE TO HAART

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In a subset of HIV-infected patients receiving combination antiretroviral therapy (HAART), here called CD4-Low Responders (CD4-LR), the CD4 counts fail to rise rapidly despite effective control of plasma viral load. CD4-LR patients ($n = 13$) included in the study had been receiving stable HAART for > 19 months resulting in undetectable viral load (< 50 copies/ml) but had < 200 CD4 lymphocytes/mm³. The mechanism responsible for the failure found in CD4-LR patients was investigated. CD4 T lymphocytes from these patients under-expressed the anti-apoptotic molecule Bcl-2, and were more susceptible to spontaneous apoptosis. Peripheral CD4 T lymphocytes from CD4-LR patients showed a regulatory dysfunction in the IL-2R system that resulted in a lack of reactivity to IL-2. Altogether, our study characterises the defective maintenance of peripheral CD4 T lymphocytes in CD4-LR patients. This group of patients received IL-2 immunotherapy in addition to HAART.

After only three cycles, their CD4 counts rise from 123 (104;134) to 229 (176;244) and reach > 512 (408;642) cells/mm³ after 8 cycles. An increase in Bcl-2 expression and IL-2 reactivity was observed in the CD4 T lymphocytes of CD4-LR patients receiving IL-2 and this correlated with a reduction in their apoptosis. Finally, we show that the magnitude of the CD4 count response correlates with the baseline expression levels of the anti-apoptotic molecule Bcl-2.

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Novel Mechanism for T Cell Apoptosis via CD28 and Protein Kinase C Activation.

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For optimal activation of naïve T cells, CD28 mediated costimulation is important, however, signals from this pathway don't always result in T cell proliferation. Indeed, CD8+ T cell apoptosis in HIV can be reduced by monocyte removal as well as by mAbs to CD80 and CD86, but not CD40 or FasL (Clin Immunol 90:302-312, 1999). Recent work shows that mAb to CTLA-4 reduced apoptosis in only 1 of 5 experiments, however, three different anti CD28 mAbs were capable of reducing apoptosis of CD8+ T cells from HIV infected patients (11 of 13 experiments). Interestingly, a different CD28 mAb induced apoptosis in resting T cells as well as in the T cell line, Jurkat, but not in other cell types. To determine how a costimulatory signal might induce apoptosis by monocytes in HIV or by an antibody to CD28, we used various kinase inhibitors. B15, a protein kinase C inhibitor, but not H89, a protein kinase A inhibitor reduced apoptosis of CD8+ T cells from HIV patients (15 of 15 experiments). B15, but not H89 also eliminated Jurkat apoptosis caused by the CD28 mAb. These data suggest that some types of CD28 stimulation induce apoptosis in mature T cells and that protein kinase C must be activated for apoptosis to occur. Because patient monocytes express increased levels of CD80 compared to controls and CD80 induces more CD28 downregulation than CD86 in Jurkat T cells, our hypothesis is that the strength or conformation of the CD80 signal influences apoptosis. Revealing the mechanism for differential behavior of T cells after CD28 stimulation may be fundamental for understanding loss of CD8+ T cell function in HIV.

LYMPHOID CELL GROWTH AND DIFFERENTIATION (238-241)

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Characterization of a novel Ly49 promoter that is active in immature cells

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The Ly49 family of receptors has been shown to play a role in the regulation of cytokine and chemokine production by murine NK cells. Analysis of the Ly49a, c, i and j promoters have identified a tissue-specific promoter adjacent to the previously defined first exon. The Ly49a promoter contains TCF-1 sites and an ATF-1 site that have been shown to play a role in gene activation and transcription. The present study reveals the presence of an additional upstream Ly49 promoter in the intergenic region preceding several Ly49 genes that code for inhibitory receptors. The upstream promoter in Ly49e produces transcripts containing an alternate exon 1 (-1a), and in the case of Ly49g, this promoter leads to the production of transcripts containing an alternate exon 1 plus a novel non-coding exon (-1b). The alternative promoter is active in bone marrow, freshly isolated liver NK cells and the murine NK cell line, LNK, but it does not function in sorted NK T cells, IL2-cultured NK cells or the EL-4 T cell line, even though these cells express Ly49g. This promoter may therefore represent an "early" Ly49 promoter that functions in the initial activation of Ly49 family members.

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ANTI-IL-4 AND ANTI-IL-13 SPECIFIC HYBRIDOMA CELLS GENERATED IN CYTOKINE-DEFICIENT MICE SHOW ABSOLUTE DEPENDENCE OF IL-6 AND PRODUCE LOW AFFINITY IgMκ MONOCLONAL ANTIBODIES

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Because IL-4 and IL-13 share common alpha chain for their receptors, they have a lot of common functional properties and participate in B-cell development and Ig isotype switching. In this work we studied possibility of induction of immune response to murine IL-4 and IL-13 and generation of anti-cytokine mAbs using IL-4KO and IL-13KO mice. Mice were immunized 2-6 times with murine recombinant IL-4 (1, 5, 10 and 20µg per injection) or IL-13 (20 and 30µg per injection) in complete and incomplete Freund's adjuvant. Ig serum titer was measured with ELISA on day 7 after each immunization series. Spleen cells were fused with P3-X63.Ag8 myeloma cells at different Ig serum titers and cloned in presence of IL-6-containing thymic stroma cell supernatant. At doses 1-10µg of IL-4 Ig serum titer was very low or undefinable. The minimal doses of IL-4 or IL-13, which could induce significant anti-cytokine Ig response with titer 5000-10000, were 20µg per injection at total dose 100µg. The optimal result (titer 10240-40960) was reached at 3-time immunization of IL-13KO mice with 30µg of IL-13 per injection. Anti-IL-4 mAbs were generated at Ig serum titer 400. At this, only 0.5% of primary hybridoma clones were anti-IL-4 positive. Anti-IL-13 cell fusions were successful at titers 20480 and 40960 (about 50% of the primary clones were positive). Both anti-IL-4 and anti-IL-13 specific hybridoma clones required the IL-6 presence at primary selection, cloning and large-scale culturing and produced low affinity IgMκ mAbs. These data demonstrate that IL-4 and IL-13 deficient mice are able to develop high polyclonal immune response to "syngenic" murine cytokines but fail generation of high affinity mAbs. Acknowledgments. Author thanks F. Finkelman and S. Morris for helping in the work.

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Role of cytokine and intracellular adaptor protein in the B cell development and differentiation.

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Hematopoietic stem cells (HSCs) give rise to variety of hematopoietic lineages and are responsible for blood production throughout adult life. Recent studies have demonstrated the potential of HSCs to produce not only all hematopoietic cells, but also various nonhematopoietic cells. Amplification of HSCs represents a potentially powerful approach to the treatment of various blood disorders, to the regeneration of damaged nonhematopoietic tissues, and to applying gene therapy by bone marrow transplantation. Lnk is an adaptor protein that is expressed preferentially in B-cells. We demonstrated that the *lnk*^{-/-} mice showed enhanced production of pro-B cells in the bone marrow that resulted in part from hypersensitivity of precursors to SCF. Here we show that Lnk is also expressed in hematopoietic precursor cells in bone marrow, and that in the absence of Lnk, the number and the hematopoietic ability of precursor cells are significantly increased. Augmented growth signals through c-Kit partly contributed to the enhanced hematopoiesis by *lnk*^{-/-} cells. Lnk was phosphorylated by and associated with c-Kit, and selectively inhibited c-Kit-mediated proliferation by attenuating phosphorylation of Gab2 and activation of MAPK cascade. We will discuss roles of Lnk in the expansion and function of HSCs.

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Role of Infiltrating Neutrophils in the Thymus after Whole-body X-irradiation

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Our previous study demonstrated that apoptosis in the thymus induced by whole-body X-irradiation is associated with transient infiltration of neutrophils. In this study the role of infiltrating neutrophils was investigated in mice rendered neutropenic by intraperitoneal injection of a monoclonal antibody, RB6-8C5. Neutropenic mice showed an increase in late apoptotic cells and a decrease in cell viability compared with normal mice 18 hours after X-irradiation. Neutropenic X-irradiated mice also showed reduced regeneration of thymus in terms of differentiation, cell number and histochemistry 7 days after X-irradiation. This effect was not seen when the mAb was injected 1 day after X-irradiation, suggesting the importance of transiently infiltrating neutrophils for thymic regeneration. In contrast, the thymus of both neutropenic and normal mice had regenerated to similar extents by 14 days. Therefore, infiltrating neutrophils may play an auxiliary role in the phagocytosis of apoptotic cells and thymic regeneration in X-irradiated mice.

HEMATOPOIETIC CELL GROWTH AND DIFFERENTIATION (242-244)

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STIMULATORY EFFECT OF ENTEROCYTE-DERIVED GM-CSF ON PROLIFERATIVE ACTIVITY OF HAEMATOPOIETIC PRECURSORS IN VITRO.

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Gut is the first local haematopoietic organ in phylogenesis, which combined all hematopoietic lineages. In murine embryo studies, it has been shown that several haematopoietic cytokines are expressed in mouse fetal intestine in the same fashion as in fetal thymus and liver, the latter both known as haematopoietic tissues. And it is known, that CD34+ progenitors located in the murine small intestine mucosa. Taken together, these data suppose, that intestinal epithelial cells (IEC) can act as a cell source of growth factor(s) that effect on early haematopoietic precursors. In present research we studied the influence of serum-free murine IEC supernatants (SIEC) on murine haematopoietic stem cells (HSC) proliferative activity by cell-suicide method based on selective death of cells at DNA-synthesis stage. The bone marrow cell pre-incubation with medium containing 20% SIEC before transplantation to lethally irradiated animals led to the proliferative stimulation of HSC. Then SIEC was separated into three fractions (F): F1 (< 30kDa), F2(10-30kDa) and F3(< 10kDa). Stimulation activity of F1 was compared to native SIEC, F2 had less activity and F3 had no up-regulate proliferative activity. Determination of haematopoietic cytokines in SIEC and fractions was measured by the electrochemiluminescence method using poly- and monoclonal antibodies. Production of Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) was detected in SIEC (37241 pg/ml), in F1 (28305 pg/ml) and in F2 (5490 pg/ml). The presence of GM-CSF in two fractions was a result of varying degrees of glycosylation. The stimulatory effect of F1 and F2 could be blocked by pre-incubating with anti-GM-CSF antibody. Thus, we found that freshly isolated murine IECs supernatants contain GM-CSF, which stimulated early haematopoietic precursors proliferation in vitro. This findings suggest that the gut epithelium may provide a suitable environment to maintain appropriate early haematopoiesis by GM-CSF production.

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Serial analysis of gene expression in human monocyte- derived dendritic cells

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Dendritic cells(DCs) play a key role in immune system. Recently, it has been reported that macrophage and DCs share a common progenitor. Human DC has been generated from CD34+ precursor cells and also blood monocytes in the presence of GM-CSF, TNF- α and IL-4. Moreover, DCs become mature form expressing CD83 by stimulating TNF- α , CD40 ligand, LPS, or monocyte-condition medium. However, the process of differentiation from monocytes to DCs has not been systematically explored. In order to define molecularly the differentiation from monocytes into DCs, we conducted serial analysis of gene expression (SAGE) study to allow quantitative analysis of an extremely large number of transcripts in human immature and mature DCs cultured with GM-CSF, IL-4, and LPS. Many of the genes that were differentially expressed in DC encode protein related to cell structure, chemokines, and cell motility. Moreover, we analyzed the gene expression in monocyte derived Langerhans like cells. It has been reported that Langerhans cells (LCs) differentiation may be induced from monocytes in response to GM-CSF, IL-4, and TGF- β . Human LCs share CD1a antigen expression with DCs generated in vitro. LCs specifically express the epithelial antigen E-cadherin, the skin homing antigen and Langerin. A total of 56,695 tag sequences from a LC library represented 21,270 different transcripts. The most expressed gene was identified to be CD74 antigen. Most of the transcripts in LCs were very similar to immature DCs. Many of the genes that were differentially expressed in LC encode protein related to enzymes such as DNase I-like 3, RNase A, serine and matrix metalloproteinases (MMPs) \bar{u} A and a tight-junction protein, Claudin 1. The identification of specific genes expressed in human DC provides novel methods to define DC subsets, and maturation and activation stage of cells of DC lineage, and also lead better understanding of DC biology.

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Stat5-independence and altered signal transduction cascades in chronic myelogenous leukemia (CML) cells selected for Gleevec resistance

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Leukemias and other tumors of hematopoietic origin are often associated with constitutive, unregulated or aberrant activation of cytokine signaling cascades. In CML and other hematopoietic disorders, Stat 5 activation is driven by BCR-ABL expression, an unregulated tyrosine kinase expressed as a consequence of 9:22 chromosomal translocation. Inhibition of BCR-ABL with Gleevec, a tyrosine kinase inhibitor approved for clinical use in CML, results in inhibition of Stat 5 activation, reduction in bcl-xL expression and the onset of apoptosis. In K562 cells selected for Gleevec resistance (K562-R), BCR-ABL expression persisted but Stat 5 activation was not detected and BCR-ABL inhibition did not affect bcl-xL expression. An altered form of Stat 5 protein (p48) was expressed in these cells and its characteristics and signaling properties are currently being evaluated. Other cytokine-mediated signaling cascades (NF- κ B, Stat 1, Stat 3, MAPK, PI3K) were unaltered in Gleevec resistant K562 cells and inhibitors of these cascades did not alter K562-R cell growth or survival. However, K562-R cells overexpressed an activated form of the src-family tyrosine kinase LYN and its selective inhibition (PP2) induced apoptosis in K562-R, and to a lesser extent, parental K562 cells. LYN and related kinases are expressed in hematopoietic cells and appear to be important in cytokine and stress-activated signaling. Together, these results suggest that leukemic cells can become Stat activation independent and upregulate pathways that constitute other elements of cytokine signaling.

LYMPHOID-MEDIATED ANGIOGENESIS

(245)

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Macrophage-tumor cell interaction in regulation of tumor angiogenesis.

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Our hypothesis focuses on the possibility that macrophage-tumor cell interaction regulates tumor angiogenesis. Monocytes are recruited to the site of a tumor by chemotactic cytokines like monocyte chemotactic protein (MCP)-1 that are released by tumor cells. The monocytes then differentiate into macrophages, most likely with the help of macrophage colony stimulating factor (M-CSF). To explore the possibility that macrophages recruitment, differentiation and activation regulates angiogenic factors production, we examined the expression of interleukin-8 (IL-8) and vascular endothelial growth factor (VEGF) in supernatants harvested from monocytes treated with MCP-1 and M-CSF and activated by bacterial lipopolysaccharide (LPS). We report that monocytes secrete low basal levels of IL-8 and VEGF protein and mRNA. Stimulation of the monocytes with lipopolysaccharide (LPS) caused an increase in IL-8 and VEGF protein secretion and mRNA expression. Treatment of monocytes with M-CSF or MCP-1 resulted in differential up-regulation of IL-8 and VEGF protein expression and mRNA expression. In addition, treatment of the monocyte-derived macrophages (MDM) with LPS resulted in increased IL-8 and VEGF protein expression. MCP-1 pretreated MDM produced lower levels of IL-8 and VEGF protein and higher levels of IL-8 mRNA compared to M-CSF treated MDM. The data suggest following treatment of MDM with MCP-1 or M-CSF. To examine the extent, to which macrophages regulate the expression of angiogenic factors in tumor cells, we treated melanoma cells (A375P) with macrophage-derived supernatants. After washing and re-feeding them, we harvested their supernatants and analyzed them for IL-8 and VEGF expression using ELISA. We observed increased production of IL-8 and VEGF in melanoma cells treated with macrophage-conditioned media. In summary, our data demonstrate that monocytes/macrophages differentially produce angiogenic factors during recruitment, differentiation and activation and regulate the expression of angiogenic factors in melanoma cells providing and emerging role for monocytes and MDM in the regulation of tumor angiogenesis.

ANTIGEN PROCESSING AND PRESENTATION (246-247)

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Physical and Functional Loss of NKT Cells Following a Vaccinia Virus Infection

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Murine CD1d1 molecules are nonpolymorphic major histocompatibility complex class I-like glycoproteins that are recognized by a unique subpopulation of T cells designated NKT cells. The role of CD1d1 molecules and NKT cells in antiviral immunity has not been extensively analyzed. In this study, we examined the effect of a vaccinia virus (VV) infection on the functional cell surface expression of CD1d1 molecules, vis-a-vis recognition by NKT cells. It was found that VV rapidly reduced NKT cell recognition of CD1d1 molecules in a time-dependent manner as measured by cytokine release. This reduction was not the result of a decrease in CD1d1 cell surface expression or the death of the CD1d1+ antigen presenting cells caused by the virus infection. It required active viral replication, as the loss of recognition was not seen in cells treated with UV-inactivated virus. In addition, we found that following a VV infection in vivo, NKT cells were lost from the liver. Collectively, our data suggest that VV-induced alterations in the qualitative expression of CD1d1 molecules result in the physical and functional loss of NKT cells. These results may have major implications in the host's innate immune response against VV and other viruses.

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A single chain Fv anti-CD64:ovalbumin fusion protein augments antigen presentation and results in higher IgG2a production.

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Receptors for the Fc portion of immunoglobulin G (FcγR) provide a crucial link between humoral and cellular immunity. In addition to effector functions such as cytokine synthesis, ADCC and phagocytosis, FcγR mediate the uptake of antigens via immune complexes and signal the maturation of dendritic cells. CD64 (the high affinity Fcγ receptor) is unique among Fc receptors in that its expression is restricted to professional antigen presenting cells (monocytes, macrophages and dendritic cells), making it an attractive target for vaccine delivery. In the past, antigens have been targeted to CD64 by either chemically or genetically fusing the antigen to whole or F(ab')₂ fragments of anti-CD64 antibodies, resulting in augmented presentation by MHC II. We have now developed a single chain Fv anti-CD64:ovalbumin fusion protein (22:OVA) that binds to CD64 and is blocked by the parent antibody, M22. In vitro, 22:OVA is presented 1,000 times more efficiently than native OVA. In vivo, immunizing with 22:OVA results in higher OVA specific Ig than does immunizing with native OVA. In addition, 22:OVA appears to skew the immune response towards a Th1 phenotype as it results in a higher OVA specific IgG2a/IgG1 ratio. Thus, single chain Fv fusion proteins provide another effective way to target antigens to CD64 positive antigen presenting cells and may provide a useful tool in shaping the Th1/Th2 balance of the immune response.

LEUKOCYTE-ENDOTHELIAL INTERACTIONS (248-249)

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IL2 CONTROLS O-GLYCAN BRANCHING AND SELECTIN LIGAND FORMATION IN CD8 T CELLS

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T cell activation has been previously associated with induction of enzymes required for selectin ligand synthesis including the O-glycan branching enzyme Core 2 (C2GnT) and fucosyl transferase FucT VII. Studies focusing on regulation of selectin ligand expression in CD4 Th1 and Th2 cells have indicated that Th1 cells bind P-selectin whereas Th2 cells do not. Differences in P-selectin ligand synthesis have been uniformly ascribed to the differential expression of FucT VII driven by Th1 polarising conditions. IL12 and TGFβ have been found to support FucTVII expression while IL4 was inhibitory for fucosylation and formation of P-selectin ligand formation. We have found evidence that CD8 T cells activated without exogenous cytokine by either Con A or [peptide + MHC] failed to induce C2GnT and P selectin ligand expression and have established a simple culture system where activated CD8 T cells become receptive to rapid induction of C2GnT and P-selectin ligand expression. Using this culture system we sought to identify factors that regulate expression of C2GnT and P selectin ligand in activated CD8 cells. We have found that exogenous IL2 added 2 days after T cell activation is sufficient to support C2GnT activity and P-selectin ligand formation in CD8 T cells. In contrast IL15 supported only moderate C2GnT induction while IL4 failed to induce C2GnT activity and neither cytokine supported P-selectin ligand formation. Thus C2GnT and selectin ligand expression appear to be regulated by an IL2-dependent process in activated CD8 T cells, suggesting that the milieu of activation can influence adhesion systems that dictate lymphocyte homing properties of the resulting activated T cells. *Supported by the Canadian Institutes of Health Research*

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SELECTIVE INHIBITION OF ENDOTHELIAL ACTIVATION BY INTERLEUKIN-10.

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Interleukin (IL)-10 is generally regarded as an anti-inflammatory cytokine, since it acts on a variety of cell types to suppress production of pro-inflammatory mediators. In this study, the actions of IL-10 on human umbilical vein endothelial cells (EC) were investigated. We found that IL-10 inhibits pro-inflammatory activation of EC, as IL-10 reduces migration of monocytes and T lymphocytes across monolayers of EC stimulated by lipopolysaccharide (LPS). IL-10 also decreases endothelial production of the chemokines IL-8 and monocyte chemoattractant protein 1 in response to both LPS and *Borrelia burgdorferi*, the causative agent of Lyme disease. However, IL-10 does not affect these responses when EC are activated by the host cytokines IL-1β or tumor necrosis factor α (TNFα). IL-10 reduces production of IL-8 in response to *B. burgdorferi* over a time period of at least 48 hours, with a concentration of 2 ng/ml eliciting maximum effects. Pretreatment of EC with IL-10 prior to their stimulation has no effect; however, IL-10 is able to suppress secretion of IL-8 even when added up to 4 hours after exposure of EC to *B. burgdorferi* has begun. Surprisingly, IL-10 does not prevent upregulation of the adhesion molecules E-selectin and intercellular adhesion molecule 1 by EC exposed to *B. burgdorferi*, LPS, IL-1β, or TNFα. These data suggest that IL-10 has selective actions on endothelium with regard to both stimulus and effector responses. In particular, IL-10 reduces pro-inflammatory activation of endothelium in response to bacterial stimuli, but not host-derived cytokines.

CELL-MATRIX INTERACTIONS (250-251)

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Galectin-3 Binds to Gelatinase B from Human Neutrophil Leukocytes

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Galectin-3 is a mammalian lectin with affinity for β-galactoside-containing glycoconjugates and preferential binding to poly-N-acetyl-lactosaminoglycans. Secreted by macrophages extracellular galectin-3 may bind to other inflammatory cells and modulate or activate different cellular functions, such as oxidative burst in neutrophil leukocytes. In the present study galectin-3 ligands from neutrophils were isolated by affinity chromatography and analyzed by MALDI-TOF mass spectrometry. A major band corresponding to 92 kD protein was identified as gelatinase B. Gelatinase B is a glycoprotein, and the glycans represents 15% of the total mass of the glycoprotein. The major structures of these oligosaccharides were recently reported, and those expected to bind galectin-3 were found among them. Galectin-3 may, hence, cross-link gelatinase B to other glycoproteins which it binds, e.g. at the neutrophil cell surface or laminin in basement membranes, thereby restricting the diffusion of the gelatinase to its intended substrates in the extracellular matrix near the migrating neutrophil, and preventing uncontrolled activity by gelatinase B released from neutrophil granules. Thus, the present study suggests that galectin-3 may not only directly activate neutrophils, but may also modulate inflammation through the interaction with proteins released from the neutrophils. Furthermore, these studies will provide a basis for exploring the biomedical usefulness of galectin-3 and inhibitory ligand analogs.

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MODIFICATION OF BONE MARROW ENVIRONMENT BY TGF- β ENHANCES SURVIVAL OF THE LEUKEMIC CELLS

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Interaction between leukemic cells and bone marrow stroma is a critical step regulating survival and homing of the neoplastic cells. This process is regulated by integrins and by cytokines such as transforming growth factor-beta (TGF- β) and may give malignant cells survival advantages through modification of the adjacent stroma. Hairy cell leukemia (HCL) which is characterized by excessive bone marrow fibrosis represents an ideal model to investigate these interactions. Immunoassays revealed that TGF- β is present at high concentrations in BM plasma of HCL patients. Immunofluorescence demonstrated an intense staining for TGF- β in hairy cells (HC), indicating that they are the major source of this cytokine. Subsequent in vitro studies showed that BM fibroblasts (BMF) of HCL patients produce high amounts of collagen and reticulin fibers which is further increased by addition of TGF- β or BM plasma of HCL patients. The results were confirmed by intracellular staining of procollagen type I(a1), type III (a1) and fibronectin and by RT-PCR analysis. Co-culture experiments indicated that TGF- β enhances adhesion of HC to BMF and that anti-TGF- β antibodies inhibit this process. Antibodies against α 5 β 1 integrin (VLA-4) or its ligand VCAM-1 are also able to block HC adhesion. As demonstrated by propidium iodide staining and FACS analysis adhesion to BMF is associated with increased viability of the malignant cells. These results point to a key role for TGF- β and adhesion molecules in regulating the interaction between the leukemic cells and BM fibroblasts.

CELL-CELL INTERACTIONS IN THE IMMUNE RESPONSE (252-256)

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EFFECT OF MOUSE UTERINE STROMAL CELLS ON EPITHELIAL CELL TNF α AND TGF β PRODUCTION AND TRANSEPITHELIAL RESISTANCE (TER) IN CULTURE

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Previous studies from our laboratory have shown that the mucosal immune system is present in the female reproductive tract and under hormonal control. With the recognition that underlying stromal cells regulate epithelial cell function, the current study was undertaken to more fully define cell-cell communication in the uterus and to determine the role of uterine stromal cells in regulating epithelial cell monolayer integrity, as well as TGF β and TNF α production. Uterine epithelial and stromal cells from adult intact mice were isolated by enzymatic digestion, cultured separately, and grown to confluence. After epithelial cells reached confluence on Millicell chambers, as indicated by high TER, cells were transferred to plates containing stromal cells to begin co-culture. Supernatants collected from the apical chambers were assayed for TGF β and TNF α via bioassay and ELISA, respectively. TER was monitored with an EVOM voltohmmeter. TGF β and TNF α were produced by both epithelial and stromal cells in culture. Co-culture of epithelial cells with stromal cells led to a decrease in TNF α apical release compared to epithelial cells alone. This decrease in cytokine release was maintained following stromal cell removal. In contrast, TGF β release was not effected by the addition of stromal cells. TER measurements increased with stromal cell co-culture and remained elevated following removal of stromal cells. These studies indicate that uterine stromal cells modulate epithelial cell release of TNF α and transepithelial resistance, while not effecting TGF β release. This work demonstrates that uterine stromal cells exert a differential effect on epithelial cells and play a precise role in regulating uterine integrity and epithelial function. Knowledge of epithelial-stromal interactions in the uterus is necessary to develop a better understanding of the mucosal immune system in the female reproductive tract. Supported by AI-13541 from NIH.

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Effect of Rapamycin on the Cyclosporin A-Resistant CD28-mediated Co-stimulatory Pathway

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Outcome of T cell activation depends exclusively on co-stimulation during antigen-T cell receptor interaction. Interaction between the T cell co-receptor CD28 and its ligand B7 during antigen-antigen receptor engagement results in full activation of T cells, the outcome of which is proliferation and effector functions. The ability of CD28 to co-stimulate the production of T cell growth factor interleukin-2 (IL-2) explains the importance of this co-stimulation. The signaling event mediated by CD28 engagement has been proposed to have two components: one is sensitive to the immunosuppressive drug cyclosporin A (CsA), and the other one is CsA-resistant. In this report, we demonstrate that the CsA-resistant pathway is sensitive to the immunosuppressive drug rapamycin. Treatment with rapamycin blocked IL-2 production after activation of human peripheral blood T cells with phorbol ester (PMA) and anti-CD28 (CsA-resistant pathway), whereas this drug did not have any effect on PMA plus ionomycin stimulation (CsA-sensitive pathway). The inhibitory effect of rapamycin was on IL-2 translation and the mRNA stability, rather than on the IL-2 transcription.

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Feline Immunodeficiency Virus (FIV) Infection Induces B7 \cdot CTLA4 \cdot T Cell Apoptosis: A Model for T Cell Depletion and Immunodeficiency

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The B7.1 and B7.2 co-stimulatory molecules on APC provide the necessary second signals for regulating T cell immune responses by sequential engagement of CD28 and CTLA4 on T cells. While CD28 expressed on naïve T cells transduces a signal for immune activation, CTLA4 expressed only on activated T cells transduces a signal for anergy/apoptosis, thus terminating the immune response. As T cell apoptosis and immunodeficiency are characteristics of FIV-infected cats, we speculated that B7-CTLA4 negative T cell signaling may be favored in these cats. Three-color flow cytometry analysis of lymph node (LN) T cells from cats infected with FIV revealed a progressive increase in B7.1 and B7.2 positive CD8 \cdot cells with time after infection, such that 65-95% of CD8 \cdot cells were B7 \cdot in cats with AIDS. Analysis of CD4 \cdot cells also revealed increased numbers of B7 \cdot cells in FIV \cdot cats. Dual staining for B7.1/B7.2 and CTLA4 revealed that a large fraction of B7.1 \cdot /B7.2 \cdot T cells co-expressed CTLA4. Three-color flow cytometry with anti-B7.1/B7.2, anti-CTLA4 and TUNEL analysis revealed that apoptosis was a characteristic of B7.1 \cdot B7.2 \cdot CTLA4 \cdot T cells. These data support the hypothesis that LN apoptosis and progressive deterioration of T cell immunity in FIV-infected cats is the result of chronic B7.1/B7.2-CTLA4 mediated T-T interactions leading to T cell anergy and apoptosis.

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B7.1/B7.2 and CTLA4 Expression on Feline T Cells In Vitro Correlates with Apoptosis

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T cell apoptosis in lymph nodes (LN) of feline immunodeficiency virus (FIV)-infected cats is associated with the up-regulation of B7.1 and B7.2 co-stimulatory molecules on CD4 \cdot and CD8 \cdot cells. We hypothesize that FIV induces an expansion of activated LN T cells expressing B7.1/B7.2 and

CTLA4, allowing for B7⁺ - CTLA4⁺ T cell interactions resulting in anergy and apoptosis. We developed an in vitro model whereby B7 and CTLA4 expression can be measured in parallel with T-cell apoptosis. PBMC were cultured with and without T cell mitogen, and expression of B7.1/B7.2 and CTLA4 on CD4 and CD8 cells determined by 2- and 3- color flow cytometry. B7.1 showed a relative high expression at 24 hr that remained high at 48 and 72 hr. The increase in B7.1 expression coincides with an up-regulation in B7.1 mRNA transcription as measured by semi-quantitative RT-PCR. B7.2 and CTLA4 expression was low on unstimulated T cells, increasing significantly only after 48 to 72 hr after stimulation. No increase in B7 or CTLA4 expression was observed on PBMC cultured in the absence of mitogen. Apoptosis as measured by fluorescent dTdt TUNEL staining of PBMC showed marked increase of TUNEL⁺ CD4 and CD8 cells after 24 hr of stimulation. TUNEL⁺ cells expressed B7 and CTLA4. Analysis of LN cells and PBMC of FIV⁺ cats revealed that CTLA4⁺ T cells co-expressed B7.1 and B7.2, supporting a model of bi-directional killing of activated T cells.

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T Cell Apoptosis in FIV-infected Cats is Blocked by the Addition of Antibodies to B7.1 and B7.2

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Human immunodeficiency virus (HIV) and feline immunodeficiency virus (FIV) cause lymph node (LN) T cell apoptosis that correlates with development of immunodeficiency. However, the mechanism(s) mediating T cell apoptosis and immunodeficiency remain controversial. Previous work showed that FIV-infected cats had an increased percentage of LN T cells that express B7 and CTLA4 molecules, compared to age-matched controls. Moreover, apoptosis of CD4 and CD8 LN cells from FIV⁺ cats was associated with the cells expressing B7.1/B7.2 and CTLA4. We hypothesized that interactions between B7.1/B7.2 and CTLA4 expressing T cells would transduce a signal to inhibit IL-2 production, resulting in T cell anergy in FIV-infected cats. To address this hypothesis, we developed an in vitro LN culture system to assess factors that might affect apoptosis. Two-color flow cytometry and TUNEL staining revealed that cultured CD4⁺ and CD8⁺ cells from FIV⁺ cats that expressed B7.1⁺/B7.2⁺ and CTLA4 had a high frequency of spontaneous apoptosis. Addition of antibodies to B7.1 and B7.2 to the LN cell cultures from FIV⁺ cats inhibited T cell apoptosis. Addition of IL-2 also inhibited T cell apoptosis, suggesting that IL-2 suppression mediated by B7-CTLA4 signaling may account for the increased apoptosis seen in FIV-infected animals. The FIV-infected cat may provide a useful animal model to explore the role of B7-CTLA4 signaling in HIV-associated immunodeficiency.

Th1/Th2 CYTOKINES (257-266)

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Circulating T cells in individuals living in areas with high prevalence of infections are poised to produce Th2 cytokines

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Persistent immune activation has been suggested to affect the subset composition and activation status of peripheral blood cells. In this study we have compared peripheral blood mononuclear cells (PBMC) from a group of Ghanaians living in an area with high prevalence of malaria, mycobacteria, EBV and helminthic infections to a group of European counterparts. Our hypothesis was that persistent challenge with microorganisms is associated with increased production of cytokines and increased susceptibility of periphery cells to undergo apoptosis. We observed an increased frequency of

activated T cells and a higher frequency of IL-4, but not IFN-gamma, producing cells in the periphery of the Ghanaians. The IL-4 was mainly produced by CD4⁺ cells, in contrast to IFN-gamma which was equally produced by CD4⁺, CD8⁺ and TCR-gamma/delta cells. The frequencies of cytokine producing cells were highly correlated to the frequencies of activated cells. Finally, cells from Ghanaians were more susceptible to activation induced apoptosis. These results may explain why some epidemic diseases seem to have different mode of transmission in Africa compared to the Western world, and may thus be of importance when vaccine strategies is considered in Africa.

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DIFFERENT THERAPEUTIC RESPONSES AFTER ANTI-INTERFERON (IFN)-GAMMA THERAPY IN TH1 (RHEUMATOID AND PSORIATIC ARTHRITIS) AND TH1/TH2 DISEASE (SYSTEMIC LUPUS ERYTHEMATOSUS (SLE))

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Purpose: To assess efficacy and tolerability of anti-IFN-gamma therapy in patients (pts) with rheumatic diseases Methods: Fifty-four pts with active rheumatoid arthritis (RA) (45), psoriatic arthritis (PA) (5) and SLE (4) received IM injections of antibodies to IFN-gamma, tumor necrosis factor-alpha (TNF-alpha), or placebo for 5 consecutive days. Pts were clinically assessed daily for 7 days, then weekly up to the 28th day. Clinical, laboratory, and ultrasound indices were used to evaluate treatment efficacy. Results: Anti-IFN-gamma therapy provided rapid and statistically significant reduction of joint inflammation in RA pts. The results obtained were superior to placebo and comparable to anti-TNF-alpha. The thickness of the inflamed synovial membrane assessed by ultrasound decreased significantly only with anti-IFN-gamma (both by the 7th and by the 28th days). Three pts receiving anti-IFN-gamma achieved remission of 4-60 months, and one receiving anti-TNF-alpha, 7 months. Articular and skin manifestations improved in all 5 pts with PA (2 with remissions of 30 and 48 months). In 4 pts with SLE, anti-cytokine therapy (2 with anti-IFN-gamma alone, 2 with both anti-IFN-gamma and anti-TNF-alpha) was ineffective, and 3 of the 4 pts deteriorated (2 anti-IFN-gamma, 1 combination). Tolerability of anti-IFN-gamma was good. The most frequent side effect was mild local dermatitis in sites of injections. Conclusion: The results obtained show that IFN-gamma may contribute to the pathogenesis of RA and PA and therefore could represent an important therapeutic target. Anti-IFN-gamma can be effectively used in treating RA (including its treatment-resistant forms) and PA. Administration of anti-IFN-gamma in SLE is not indicated.

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Detection of Intracellular Cytokines Production in Peripheral Blood CD3+T Cells of Patients with Recurrent Genital Herpes

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[Abstract] Objective To study the role of Th1/Th2 cytokines profile in the pathogenesis of recurrent genital herpes (RGH), and to find out the relationship between them. Methods A two-colour immunofluorescent staining of cell surface antigen and intracellular cytokines for flow cytometric analysis was used for CD3⁺IL-2⁺IL-10⁺IL-12⁺IFN- γ and TNF- α in CD3⁺ T-lymphocytes in activated peripheral blood mononuclear cells of patients with RGH. Results Compared to controls, patients with RGH showed an decreased number of CD3⁺ T cells⁺ P < 0.05 and number of IL-2 producing and IFN- γ -producing T cells⁺ P < 0.02 and P < 0.001, respectively after in vitro stimulation with PMA and ionomycin in the presence of a protein transport inhibitor, whereas an increased number of IL-10-producing and IL-12-producing T cells was found in patients with RGH⁺ P < 0.01. There is no significant differences in the number of TNF- α -producing cells between the patients and controls⁺ P > 0.05. Conclusion RGH patients showed a predominance of Th2 cytokine profile. The imbalance between Th1 and Th2

cytokines results in inhibitory effects on a series of cell-immune response, which may play an important role in the pathogenesis of RGH.

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IFN- α and IL-12 induce interferon regulatory factor 4 (IRF-4) and IRF-8 gene expression in human NK and T cells

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IFN- α and IL-12 are macrophage-derived cytokines that enhance innate and Th1-type immune responses. However, there is only limited information on IFN- α and IL-12 target genes mediating their immunostimulatory effects. The interferon regulatory factor (IRF) family of transcription factors is known to be involved in controlling lymphocyte differentiation and functions. In this work we have studied the effect of IFN- α and IL-12 stimulation on IRF gene expression in human NK and T cells. Both IFN- α and IL-12 strongly up-regulated IRF-4 and IRF-8 mRNA and protein expression in these cells. Also, binding of IRF-4 and IRF-8 proteins to target promoter control elements was enhanced. Following stimulation with IFN- α or IL-12, Stat4 was found to bind to a GAS element from the promoter region of the *IRF-4* gene, as detected by EMSA and DNA affinity purification, demonstrating that IRF-4 is a direct target gene of both cytokines. Our results suggest that IFN- α and IL-12 may enhance innate and Th1-type immune responses by inducing IRF-1, IRF-4, and IRF-8 gene expression.

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A proteome database of human primary T helper cells

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At present, the first versions of human genome are available, and the genomes of many model organisms are known. The genome, however, does not tell us anything about the expression levels of genes. Therefore two additional methodologies, transcriptomics and proteomics have become increasingly important. These studies produce large amounts of data, and processing, organizing, and storing of the data needs to be solved efficiently. As a crucial first step of our studies on T helper cell differentiation at the protein level we have established the first public database of human primary T helper cell proteome using two-dimensional electrophoresis (2-DE) and matrix-assisted laser desorption ionization/time-of-flight mass spectrometry. For the database, CD4⁺ human T cells were activated with anti-CD3+anti-CD28 antibodies and metabolically labeled with 35S-methionine for 24 hours. Cells were lysed and proteins were separated by 2-DE. About 1500 protein spots are detected in the resulting 2-DE gels with silver staining, and 2000 spots with autoradiography. We have identified 91 proteins from the 2-DE gels using peptide mass fingerprinting, and annotated them to our database. The identified proteins are also linked to Swiss-Prot and NCBI protein databases. Our database is soon available via the Internet at <http://www.btk.utu.fi/hotwire/Genomics>.

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Serum level of the main immunoregulation cytokines and some immunological status parameters in the population exposed to radiation influence resulted from the nuclear test action at the Semipalatinsk test site.

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An increase of the immune system diseases, including immunodeficiency disorders, allergy and autoimmune diseases was found in the population exposed to the radiation influence as the result of action of Semipalatinsk test site in comparison with the population not exposed to this influence. The changes were observed in the subpopulation structure of peripheral blood

immunocompetent cells, including an increase of CD3⁺ cells and a decrease of ND4⁺ cells and ND8⁺ cells in the people of the first generation who were directly exposed to the radiation influence. It can indicate the regulation interaction disorders in the immune system. The increase of IL-1 β and TNF- α serum level was established compared to the normative meanings. The serum levels of the proinflammatory cytokines in people, who were exposed to the radiation influence were increased along with the increase of a radiation dose, which coordinates with the increase of IL-1 β and TNF- α gene expression in peripheral blood mononuclear cells. The increase of the serum proinflammatory cytokine levels is accompanied by a significant increase of serum levels of Th1 and Th2 cytokines (IL-4 and IFN- γ). The increase of IL-1 β , TNF- α , IL-4 and IFN- γ serum levels was observed not only in the people, who were exposed to radiation influence, but also in their first and second generation offspring's. It was shown that an increase of IL-4 and IFN- γ serum levels was observed only if proinflammatory cytokines serum level was higher than the fixed threshold value. We can conclude that the changes in proinflammatory cytokine production resulted by the radiation influence appear to be the leading pathogenic influence on the cytokine network. The increase of the immune diseases in the Altai inhabitants who were exposed to radiation is accompanied by changes in the subpopulation structure of peripheral blood immunocompetent cells and also in the immunoregulatory cytokine serum level, especially in the proinflammatory cytokines.

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IL-4 Administration restores immunity in male mice following ethanol exposure and burn injury

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Ethanol exposure prior to thermal injury exacerbates the immune dysfunction that is seen after burn injury, which is mediated by increased production of IL-6. Since IL-4 is known to negatively regulate IL-6 production, the goal of the current study was to evaluate the role of IL-4 on immunity in injured mice. A 15% total body surface area burn (or sham) injury was administered to mice in the presence or absence of circulating ethanol levels of 100 mg/dl (2 hard liquor drinks in an adult male). At 30 min post-injury, mice were treated with murine IL-4 (5 ug/i.p.) or saline vehicle. The DTH response was suppressed in ethanol/burn mice by 45% in comparison to all other groups. This suppression was coincident with 25% suppression in splenocyte proliferation, a 2-fold increase in splenic macrophage-derived IL-6, and a 50% decrease in IL-4 production by splenic lymphocytes. IL-4 treatment of ethanol/burn mice completely restored the DTH response and partially restored splenocyte proliferation. Moreover, in vitro addition of IL-4 (300 pg/ml) to cultures generated from ethanol/burn mice normalized the elevated macrophage production of IL-6 production and restored splenocyte proliferation in a manner similar to what was seen with in vivo cytokine treatment. These data suggest that proper cytokine balance is essential for intact immune function after injury. (Supported by NIH GM55344, AA12034, and AA11134)

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Circulating Anti-Human Heat shock Protein 65(HSP65) IgE autoantibody in Atopic Dermatitis

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Heat shock protein 65 is an inducible protein by stress conditions and has been suggested to play a role in inflammatory diseases because it is induced by such cytokines as interleukin 2 and TNF- α . Atopic dermatitis patients show aggravation episodes by stress conditions such as infections. In this study, the possibility of presence of IgE autoantibody to human heat shock protein 65 to obtain a clue of the pathogenic mechanism for clinical aggravation episodes during stress conditions in atopic dermatitis. A total of 29 patients who fulfilled the diagnostic criteria for atopic dermatitis were selected. Anti-human heat shock protein 65 IgE autoantibody was detected in the sera of atopic dermatitis. Among 29 patients 11 (37.9%) showed anti-human heat shock protein 65 IgE autoantibody in atopic dermatitis. However, the presence of anti-human heat shock protein 65 was not exactly consistent with the clinical

aggravation episodes of febrile reaction in this study. mRNA expression of induced heat shock protein was detected by PCR and western blotting respectively. Circulating anti-human heat shock protein 65 IgE autoantibody was present in a part of atopic dermatitis as one of possible pathogenic mechanisms for the clinical aggravation episodes of atopic dermatitis.

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Synergistic Effect of IL-4 on IL-2- and IL-12-Induction of Murine IFN- γ Expression in NK Cells

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IL-4 is thought to influence T and NK cells by down-regulating Th1 type cytokines like IFN- γ . While investigating IL-4 regulation of IFN- γ expression, we found that IL-4 synergized with IL-2 or IL-12 to enhance IFN- γ production and mRNA expression in spleen derived, IL-2 cultured NK cells (LAK). In addition, negatively sorted fresh DX5+/CD3- NK cells also show IL-2/IL-4, and IL-12/IL-4 synergy on IFN- γ expression, albeit at lower levels. To determine the role of Stat 6, LAK cells from C57Bl/6 Stat 6 -/- mice displayed an almost 10-fold drop in IFN- γ production in response to IL-4 and IL-2. In contrast, IL-12/IL-4 synergy on IFN- γ expression was intact in the Stat 6 -/- mice. To explore possible molecular mechanisms to account for the synergistic effects of IL-4 on murine NK cells, we found that IL-2 + IL-4 stimulation resulted in a modest increase in tyrosine phosphorylation of Stat 5, while IL-12/IL-4 treatment resulted in a more substantial increase in tyrosine phosphorylated Stat 4. Finally, to identify regions of the IFN- γ promoter that may be involved, LAK cells from human IFN- γ promoter/luciferase transgenic mice were treated with cytokines. NK cells from proximal (-110 to +64) promoter region mice did not respond to cytokines. However, the intact -565 to +64 IFN- γ promoter responded synergistically to IL-2/IL-4 in NK cells isolated from this transgenic mouse. These data suggest a role for IL-4 in enhancing IFN- γ expression in murine NK cells that is partially dependent on Stat 6 in IL-2 co-stimulation, and completely independent of Stat 6 in IL-12 co-stimulations.

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Th1 /Th2 cytokine expression by cord blood mononuclear cells compared to adult PBMC's; the sensitive measurement by a novel cell-based ELISA assay (CeELISA).

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It was demonstrated in 1989¹ by Broxmeyer and colleagues, that umbilical cord blood (UCB) is a rich source of haemopoietic stem/progenitor cells and as such could be used in the clinical setting for haemopoietic stem cell transplantation. An additional advantage of the use of UCB stem cells for transplantation, was the observation that transplant related complications (e.g. graft-versus-host disease; GVHD) were less severe than in bone marrow transplantation (BMT)². It was suggested that since UCB comprises mainly of naïve, unprimed T lymphocytes which are predominantly suppressor cells, that an "immature" immune response would result from decreased cytokine expression from these cells, and thus contribute to the reduced incidence and severity of GVHD. Thus we have collected mononuclear cells from 60 healthy adult donors and 59 cord bloods from normal uncomplicated deliveries. Cells were cultured in the presence and absence of pokeweed mitogen (PWM) stimulation and determined for cytokine expression using a novel and highly sensitive cell-based ELISA (CeELISA)³ technique. We present data on the expression of the key Th1 and Th2 cytokines TNF α , γ IFN, IL1 β and IL10, which are all known to contain genetic polymorphisms which influence the level of gene expression. Polymorphism was determined by induced heteroduplex generator (IHG)⁴ analysis and microsatellite analysis by PCR and PAGE. We demonstrate that using these sensitive techniques, inconsistencies in current data correlating gene expression with polymorphism can be influenced by the use of unnatural mitogenic stimuli in cell culture, and that measurable cytokine expression can be achieved in unstimulated cells. We also compare overall cytokine expression for adults and cords, with and

without consideration of genetic polymorphism, and show that an overall consideration of genetic haplotype and expression "profile" may be more realistic in predicting outcome of transplantation than the correlation to expression of a specific cytokine or polymorphism. References: ¹ - Broxmeyer HE, Douglas GW, Hangoc G, Cooper S, Bard J, English D, Arny M, Thomas L, and Boyse EA (1989) Proc. Natl. Acad. Sci USA 86; 3828-3832. ² - Abecasis MM, Machado AM, Boavida G, Silva MG, Lucio P, Ambrosio A and Jorge ML (1996) Bone Marrow Transplantation 17, 891-895. ³ - Beech JT, Siew LK, Ghorraishian M, Stasiuk LM, Elson CJ and Thompson SJ (1997) J. Immunol. Methods 205, 163-168. ⁴ - Morse HR, Olomolaiye OO, Wood NAP, Keen LJ and Bidwell JL (1999) Cytokine 11, 789-795.

INFLAMMATION (267-276)

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THE NEUROINFLAMMATORY ROLE OF CAP37

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The concept that inflammation is associated with, and contributes to the pathogenesis of neurodegenerative diseases including Alzheimer's disease (AD) is gaining considerable acceptance. In particular, microglia, the central nervous system (CNS) equivalent of blood monocytes and a component of the senile plaque a hallmark of AD pathology, may be instrumental in causing neuronal injury and death. Although, it is known that activated microglia produce mediators that orchestrate cell death, the mechanisms and mediators involved in initiating or regulating these processes have yet to be elucidated. We have identified a novel inflammatory mediator, CAP37 localized to the microvasculature and neurons in AD brains. We hypothesize that CAP37 a mediator involved in the recruitment and activation of monocytes in the systemic circulation, recruits and activates microglia in the CNS thus contributing to the augmentation of AD. As an assessment of microglial activation we measured parameters known to be important in the development of the senile plaque. Microglia treated with CAP37 developed amoeboid morphology, showed strong chemotaxis, and were activated to produce superoxide anions, tumor necrosis factor- α and interleukin-1 β , two cytokines associated with AD brains. RT-PCR showed upregulation of the two chemokines, fractalkine and RANTES. Our findings demonstrate the importance of CAP37 as an inflammatory mediator in the CNS. An understanding of its mechanism of induction and mode of action in the CNS could in the long-term help determine key pharmacological points of intervention that could develop into new therapies.

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Upregulation of IL-18 and IL-12 in the Ileum of Rats with Necrotizing Enterocolitis

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Necrotizing enterocolitis (NEC) is a common and devastating gastrointestinal disease of premature infants. The major risk factors; prematurity, formula feeding, intestinal ischemia/hypoxia and bacterial colonization, may promote an inflammatory cascade that results in the pathology associated with this disease. Because proinflammatory cytokines IL-18, IL-12 and INF γ have been implicated in other diseases of the small intestine, we hypothesized that these cytokines would play an important role in NEC pathogenesis. NEC was induced in newborn rats using a combination of enteral feeding with rat milk substitute and asphyxia/cold stress (RMS). Dam fed, asphyxia/cold stressed littermates (DF) were utilized as controls. After 96 hours, distal ileum was removed from all animals and tissue was processed to determine expression and localization of IL-18, IL-12 and INF γ using real-time reverse transcription polymerase chain reaction (real-time RT-PCR) and immunohistology, respectively. In addition, disease progression was evaluated using a blinded histologic scoring system. IL-18 and IL-12 mRNA from the RMS group were increased ($p \leq 0.05$) compared to DF controls and there was a correlation

between increasing IL-18 and IL-12 mRNA levels and progression of tissue damage (Spearman rank correlation, $r = .629$ and $.588$, respectively; $p \leq 0.05$). Immunohistology revealed increased IL-18 in the cytoplasm of villi and crypt enterocytes and IL-12 positive mononuclear cells were increased with disease progression ($r = .503$; $p \leq 0.05$). No differences in the number of IFN γ positive cells were observed between groups. These data demonstrate upregulation of IL-18 and IL-12 in experimental NEC and a correlation between production of these proinflammatory cytokines and the degree of tissue damage.

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Anti-inflammatory Effect of Newly Synthesized Tumor Necrosis Factor- α , D-297

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Tumor necrosis factor(TNF)- α is a primary inflammatory cytokine usually produced in activated macrophage and is known to work as a mediator of many inflammatory disease. There is a widely accepted belief that inhibition or modification of TNF- α overproduction in different inflammatory diseases like rheumatoid arthritis and chronic asthma would be of benefit in the treatment of some of these condition. D-297, chemically synthesized novel compound, inhibited TNF- α production in vitro (LPS-stimulated murine macrophage cell line RAW264.7; IC_{50} 77 nM) and in vivo (LPS-stimulated C57BL/6 mice; ED_{50} 3.4 mg/kg, po). TNF- α mRNA expression was increased by LPS stimulation and D-297 suppressed this mRNA expression in RAW264.7 cells. Also, orally administered D-297 inhibited ear inflammation induced by arachidonic acid in mice (ED_{50} 4.7 mg/kg). These results suggest that D-297 may have anti-inflammatory action by inhibiting TNF- α production through the suppression of mRNA expression. [Supported by grants from KOSEF]

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Endotoxemia induces rapid shedding followed by upregulation of the monocyte hemoglobin scavenger receptor CD163

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CD163 is a monocyte and macrophage specific surface glycoprotein that has been reported to be a scavenger receptor for hemoglobin/haptoglobin (Hb/Hp) complexes. CD163 expression on cultured monocytes is modulated by cytokines, phorbol esters, and bacterial lipopolysaccharide (LPS or endotoxin). Recently, we have developed a CD163 enzyme linked immunosorbant assay (ELISA) and demonstrated that normal human plasma contains immunoreactive soluble CD163. We now report a rapid rise in plasma CD163 concentrations in patients undergoing coronary artery bypass graft (CABG) surgery with cardiopulmonary bypass (CPB) as well as in human subjects following intravenous LPS administration in an experimental protocol. The striking rise in immunoreactive CD163 in plasma during endotoxemia appears to be one of the earliest detectable responses to endotoxin. We also show that LPS, like PMA, causes shedding of surface CD163. Surface expression of CD163 on cultured monocytes, monitored by immunostaining and flow cytometry, decreased in parallel with an increase in immunoreactive CD163 in cell culture supernatants. Finally we demonstrate that monocyte surface CD163 increases 12-24 hours following experimental endotoxemia. This finding is consistent with increased synthesis of CD163 and may be a consequence of increased cortisol, IL-6 and IL-10 levels following LPS administration.

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A role for Interferon- γ (IFN- γ) in controlling neutrophil recruitment during peritoneal inflammation.

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We have investigated the role played by IFN- γ in controlling the phenotype of leukocytes recruited during peritoneal inflammation. I.p. inflammation was induced in wild-type (IFN- $\gamma^{+/+}$) and IFN- γ deficient (IFN- $\gamma^{-/-}$) mice using *S.epidermidis* bacterial cell free supernatants (BCFS) or IL-1 β (100ng). PMN and i.p. cytokine and chemokine levels (IL-6, MIP-2, KC,) were then assessed. I.p. injection of BCFS in IFN- $\gamma^{+/+}$ mice resulted in an early influx of PMN (mean \pm sem, $\times 10^5$), peaking at 3 hours (18 ± 3.1 ; $p < 0.05$ vs.PBS,n=6) which resolved by 6 hours. PMN influx was preceded by a peak in the levels of IL-6, MIP-2 and KC (1043 ± 151 , 682.6 ± 7.2 , 2034.4 ± 35 respectively; $p < 0.05$ vs.PBS,n=6),(pg/ml) at 1hr. In IFN- $\gamma^{-/-}$ animals the magnitude of PMN influx (mean \pm sem, $\times 10^5$) was lower at 3hrs (4.7 ± 0.7 ; $p < 0.05$ vs.PBS,n=6) and their clearance delayed. PMN levels (mean \pm sem, $\times 10^5$) were still significantly elevated compared to IFN- $\gamma^{+/+}$ mice at 6hrs (4.2 ± 0.27 and 2.7 ± 0.55 respectively; $p < 0.05$ vs.wild-type,n=6). A similar delayed profile was seen in the levels of the i.p chemokines MIP-2 and KC. In IFN- $\gamma^{+/+}$ mice in which IFN- γ (0.5-50ng) was injected together with IL-1 β (100ng), a dose dependant decrease in i.p. KC levels and a concomitant decrease in recruited PMN, was seen at 3 hours. The levels of IL-6 in these animals were significantly elevated in the presence of IFN- γ (5ng) compared to IL-1 β alone (284 ± 40 vs. 167 ± 57 respectively; $p < 0.05$ vs.PBS). Administration of IFN- γ (0.005ng-50ng) in combination with BCFS to IFN- $\gamma^{-/-}$ mice, increased the levels of PMN recruitment (7.2 ± 0.8 ; $p < 0.05$ vs. PBS),(mean \pm sem, $\times 10^5$), and delayed PMN clearance, similar to that seen in IFN- $\gamma^{+/+}$ animals. The levels of i.p chemokines MIP-2 and KC mirrored what was seen with the PMN influx. In the presence of IFN- γ , IL-6 levels were also increased and remained significantly above control at 3 hours. These findings suggest that IFN- γ is involved in controlling the recruitment and clearance of PMN during peritoneal inflammation, through regulation of resident cell C-X-C chemokine production and possibly modulation of IL-6 production.

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Regulation of leukocyte recruitment during acute inflammation by IL-6 and its soluble receptor

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During acute inflammation leukocyte recruitment is characterized by an initial influx of neutrophils, which are later replaced by a more sustained population of mononuclear cells. Here we show that IL-6 and its soluble receptor (sIL-6R) control this pattern of leukocyte recruitment during inflammation. Peritonitis is a recurrent complication in end-stage renal failure patients undergoing peritoneal dialysis and sIL-6R levels were analyzed in effluent obtained from infected individuals. When assessing sIL-6R levels, it is essential to consider that proteolytic cleavage (PC) and differential mRNA splicing (DS) contribute to the overall concentration of sIL-6R encountered. Maximal sIL-6R levels were seen on day 2 of infection and these correlated ($r=0.88$, $p < 0.05$) with the number of neutrophils infiltrating the peritoneal cavity. These levels did not however correspond with increases in DS-sIL-6R, which were highest on day 3 and coincided with the onset of intraperitoneal mononuclear cell influx. Consequently, we tested whether sIL-6R in combination with IL-6 could regulate chemokine expression by human peritoneal mesothelial cells. Both PC- and DS-sIL-6R were found to induce release of CCL2, but not CXCL1 or CXCL8. PC- and DS-sIL-6R-mediated signaling however significantly inhibited the IL-1 β -activated release of CXCL1 and CXCL8. Preferential

recruitment of mononuclear leukocytes and suppression of neutrophil accumulation by both PC- and DS-sIL-6R was confirmed *in vivo* using IL-6KO mice and a model of peritoneal inflammation that closely mimics human peritonitis. The sIL-6R isoforms are therefore important intermediates in the resolution of inflammation and supports transition between the early, neutrophilic stage of an infection and subsequent mononuclear cell influx.

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Oncostatin-M: A differential regulator of chemokine expression

Suzanne M. Hurst¹, Jamie Monslow¹, Rachel M. McLoughlin², Nicholas Topley² & Simon A. Jones¹. 1. Cardiff School of Biosciences, Cardiff University, Cardiff CF10 3US, Wales UK. 2. Institute of Nephrology, University of Wales College of Medicine, Cardiff CF14 4XN, Wales, U.K. We have recently shown that IL-6 and its soluble receptor can orchestrate a temporal switch in the pattern of leukocyte recruitment seen during acute inflammation (Hurst et al., 2001 *Immunity* 14: 705). To determine whether other IL-6-related cytokines could perform similar functions, we have examined their properties in the context of peritoneal inflammation. Peritoneal effluents were obtained from patients on peritoneal dialysis with overt clinical peritonitis, and levels of oncostatin-M (OSM) and leukemia inhibitory factor (LIF) analyzed during the first 5 days of infection. Consistent with that of IL-6, OSM was significantly increased on day 1 of infection and rapidly declined to baseline by days 2-3. No change in LIF was observed. To assess which cells within the peritoneal cavity could potentially respond to OSM, FACS analysis was performed on human peritoneal mesothelial cells (HPMC), neutrophils and mononuclear cells using antibodies for gp130 and receptors for OSM (gp160) and LIF (gp190). Although all cell types expressed gp130, gp160 was restricted to HPMC and gp190 to monocytes. Stimulation of HPMC with OSM resulted in phosphorylation of gp130 and gp160, and was associated with activation of STAT3. To assess whether OSM could induce chemokine secretion from HPMC, cells were stimulated with OSM (0.1-50 ng/ml) and CCL2, CCL3, CCL5, CXCL8 and CXCL10 release monitored. OSM only induced release of CCL2, but could also significantly suppress the IL-1-induced secretion of CXCL8 by HPMC. LIF and IL-11 had no effect on any of the chemokines tested. Gp130 and gp160 blocking antibodies (but not anti-gp190) inhibited CCL2 release. These data suggest that OSM may serve a pivotal role in suppressing neutrophil influx and promoting mononuclear cell infiltration during acute inflammation.

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IFN gamma depletion enhances inflammation and lesion progression of *Leishmania major* infected IL-4 deficient C57BL/6 mice.

Elisabet Caler, Nancy Noben-Trauth, David Sacks and Yasmine Belkaid. National Institutes of Allergy and Infectious Diseases, Laboratory of Parasitic Diseases, NIH. Infection with the intracellular protozoan parasite *Leishmania major* results in localized inflammation and lesion formation at the site of inoculation by the vector sand fly. The natural course of infection can be mimicked in the resistant C57BL/6 mouse model, by intradermal inoculation in the ear of a low dose (102) of infective parasites, resulting in cutaneous lesions that spontaneously heal. This model has helped to define the role of Th1 cells in both lesion formation and acquired resistance. In studies intended to address whether the requirement for IFN γ in controlling *L. major* growth and lesion development remains absolute even in the absence of the Th2 driving cytokine, IL-4, we investigated the effect of IFN γ depletion in WT and IL-4 KO C57BL/6 mice. While the anti-IFN γ treated IL-4 KO mice also developed severe lesions that failed to heal, the onset of the lesions and their early development (4-5 weeks) was in fact more rapid than in the untreated mice or in the anti-IFN γ treated WT mice. This *L. major* driven inflammatory process was associated with a sudden infiltration of PMNs and CD4⁺ T lymphocyte and was not due to an accumulation of greater numbers of infected macrophages, as indicated by the similar parasitic loads found at 4-5 weeks in the ears and draining nodes of WT and IL-4 KO C57BL/6 anti-IFN γ treated mice. A similar exacerbation of early lesion formation was seen in CD8⁺ T cell depleted IL-4 KO mice. The

results suggest that a delicate balance between the levels of IL-4 and IFN γ , possibly derived from CD8⁺ T cells, may be responsible for regulation of the inflammation, and that the absence of both cytokines leads to dysregulated recruitment of and/or persistence of recruited cells during the early stages of T cell-mediated inflammation in the skin.

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Altered inflammatory response in TSG-14/PTX-3 transgenic mice

Dias AAM1, Souza DG2, Diniz SN1, Gomes RN3, Goodman A4, Bozza PT3, Montagnini AL5, Vilcek J4, Teixeira MM2, Reis LFL1, 5. 1Ludwig Inst. for Cancer Res, Sao Paulo, Brazil; 2UFMG, Belo Horizonte, Brazil; 3FioCruz, Rio de Janeiro, Brazil; 4NYU School of Medicine, NY, USA; 5Hospital do Câncer, Sao Paulo, Brazil TSG-14/PTX3 is a gene inducible by TNF α , IL-1 β and LPS in fibroblasts, macrophages and endothelial cells. TSG-14 is a 42kDa protein with homology to the acute phase proteins CRP and SAP. We generated transgenic mice (TSG-14tg) overexpressing the murine TSG-14 gene under the control of its own promoter. TSG-14tg mice are more resistant to LPS-induced shock and to sepsis caused by CLP, have higher basal levels of IL-10 and, early after LPS injection, produce more TNF α than wt mice. Following ischemia and reperfusion (I/R) of the superior mesenteric artery, TSG-14tg mice showed impaired survival rate and an increased local (duodenum, ileum) and remote (lung) vascular permeability, hemorrhage and neutrophil accumulation. Moreover, after I/R we detected higher levels of TNF α in the organs and serum of TSG-14tg mice as well as a greater expression of IL-1 β and KC in the duodenum and of KC and MCP-1 in the ileum. When TSG-14tg and wt mice were injected with cerulein, both lineages developed acute pancreatitis as indicated by increased serum levels of α -amylase. However, histological analysis revealed that TSG-14tg mice have augmented pancreatic edema. Finally, macrophages from TSG-14tg mice showed an increased phagocytic capacity and produced higher levels of NO in response to IFN γ and LPS/TNF than macrophages from wt mice. Our data suggest that TSG-14 plays a critical role during the inflammatory response *in vivo*.

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Anti-IL-6 receptor antibody treatment improves interstitial lung diseases in IL-6 transgenic mice

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Interstitial lung diseases are a heterogeneous group of disorders with varying patterns of interstitial inflammation and/ or fibrosis. It was reported that transient expression of IL-6 in the lung of Wister rats induces lymphocytic alveolitis without marked fibroblast proliferation, whereas TGF- β induces fibroblast proliferation and collagen fibril deposition with only mild cellular infiltration into the alveoli. Therefore, these cytokines appears to play a significant role in interstitial lung diseases. Castleman's disease (CD) is an atypical lymphoproliferative disease with aberrant IL-6 production, and is often associated with lymphocytic interstitial pneumonia (LIP). To evaluate IL-6 transgenic mice as a model of LIP associated with CD, we histologically analyzed the lung tissue of the mice. Lymphocyte infiltration around blood vessel and bronchus was observed in the lung of IL-6 transgenic mice. However, distinct fibrotic changes were minimal. These pathological features were quite similar to that of LIP associated with CD. Then we examined the effect of anti-IL-6R antibody as a new therapeutic approach for the interstitial lung disease in this model. Administration of anti-IL-6R antibody strongly suppressed infiltration of inflammatory cells. Next, we examined whether IL-6 has antifibrogenic effects similarly to IFN- γ because IL-6 and IFN- γ share the JAK-STAT1 signal transduction pathway. IL-6 had little effect on TGF- β -induced lung fibroblast proliferation and collagen production, whereas IFN- γ suppressed both. Thus IL-6 does not seem to act as an antifibrogenic factor. Our data suggest that IL-6 transgenic mouse is a good model of LIP complicated with CD. Besides, the blockade of IL-6 signaling by anti-IL-6R antibody could be a new therapeutic strategy for LIP.

IMMUNOPATHOLOGIC MECHANISMS (277-281)

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ANTIGEN DOSE DEFINES OPPOSITE TH1/TH2-TYPE RESPONSES IN THE LUNGS OF C57BL/6 AND BALB/C MICE INDEPENDENTLY OF SPLENIC RESPONSES

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To investigate the effect of antigen dose on immune responses mediated by Th1/Th2-type cytokines, C57BL/6 and BALB/c mice were sensitized with aluminum hydroxide gel-precipitated ovalbumin (OVA) and challenged with aerosolized OVA. Low-dose sensitization (less than 8 µg of OVA) elicited Th2-type cytokines production from C57BL/6 lungs, including high levels of IL-4, IL-5, eotaxin, RANTES and a low level of IFN-γ, while BALB/c lungs generated Th1-type cytokines, including low levels of IL-4, IL-5, eotaxin and a high level of IFN-γ. In contrast, high-dose sensitization (more than 50 µg) elicited Th1-type cytokines in C57BL/6 lungs, while BALB/c lungs generated Th2-type cytokines. Furthermore, the number of eosinophils infiltrating into the lungs of low-dose OVA-sensitized C57BL/6 mice was significantly greater than in BALB/c mice sensitized with the same amount of OVA, while a very high dose of OVA (1 mg) could induce greater eosinophil infiltration into the lungs of BALB/c mice compared with C57BL/6 mice. Interestingly, splenocyte cultures from C57BL/6 mice produced Th1-type cytokines, while cultures from BALB/c mice produced Th2-type cytokines regardless of OVA sensitization dose (100 ng - 1 mg). These results indicate that C57BL/6 and BALB/c mice have different susceptibilities to OVA-induced pulmonary eosinophilia, independently of splenic Th1- and Th2-type cytokines production.

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TRANSPLANT RELATED ISCHEMIA, HIF-1α, AND FIBROGENIC GROWTH FACTORS

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In kidney transplantation, the most prevalent cause of graft dysfunction is chronic allograft nephropathy (CAN), an irreversible non-treatable process of fibrosis. A leading hypothesis is that CAN is triggered at the time of transplantation by a cascade of inflammatory responses resulting from ischemia/hypoxia. Cells exposed to ischemia produce hypoxia inducible factor (HIF)-1α a transcription factor for fibrogenic cytokines. To determine the effect of ischemia on mediators responsible for the production of cytokines involved in fibrosis of kidney transplants we analyzed the expression of HIF-1α in time zero biopsies from living donor kidneys (N=13, < 2.5 h ischemia) and cadaveric donor kidneys (N=9, 12-32 h ischemia). By real time RT-PCR analysis, we found that the HIF-1α mRNA expression level was strongly associated with the cold ischemia time. The HIF-1α mRNA expression levels in time zero biopsies of cadaveric donor kidneys was significantly higher (5-fold) than in time zero biopsies of living donor kidneys (p=0.001). In these cadaveric donor kidneys also the TGF-β mRNA expression level was significantly upregulated (p=0.05). The mRNA expression level of TGF-β was correlated with HIF-1α mRNA levels: with rising HIF-1α mRNA expression levels the TGF-β mRNA levels were increased (r=0.63, p=0.0016; Spearman's test). No effect of ischemia was seen for bFGF and VEGF165 mRNA expression levels. We found that prolonged ischemia time leads to higher mRNA expression levels of HIF-1α the fibrogenic response by triggering the production of the transcription factor HIF-1α. Our results support a role for HIF-1α in the pathogenesis of chronic allograft nephropathy.

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Estrogen replacement restores immunity in aged mice by suppressing IL-6 production

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The aged have weaker immune responses and elevated IL-6 levels than young adults. We chose to test whether estrogen replacement could lower IL-6 production and restore cell mediated immune responses after a stress, such as scald injury. To accomplish this, 3 and 18 month old mice were given E2 (17β estradiol) or placebo control prior to sustaining a dorsal scald or sham injury. Young mice could mount a robust delayed-type hypersensitivity (DTH) response regardless of injury, whereas the response was diminished in sham aged mice relative to young mice (p < 0.05), and completely absent after in aged mice injury (p < 0.01). Circulating IL-6 was undetectable in sham young mice but elevated after injury (p < 0.01). Sham aged mice had greater IL-6 levels than that of sham young mice (p < 0.05). After injury, IL-6 levels in aged mice were increased 4-fold over that of sham aged mice (p < 0.01). Injured aged mice given placebo had DTH responses that were only 21 that of young sham controls (p < 0.01). In contrast, E2 replacement in injured aged mice resulted recovery of the DTH response to 73 that of sham values (p < 0.05). This was associated with a decrease in IL-6 levels. Similar immunity was restored in injured aged mice given anti-IL-6 antibody treatment. Additional in vitro studies revealed that E2 attenuated macrophage IL-6 production and that this is a direct effect of the hormone on macrophage NF-κB activity. Thus, E2 restores immunity in injured aged mice and it is likely to involve regulation at the levels of the IL-6 promoter. (Supported by NIH GM55344 (EJK), AG16067 (EJK), AG18859 (EJK), and AG00997 (PLW))

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FOOD-SPECIFIC ORAL TOLERANCE INDUCTION FOR MILK USING INTERFERON-γ IN ATOPIC DERMATITIS

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For food-specific tolerance induction for allergy, many methods have been tried without any satisfactory results. IFN-γ/ IL-4 imbalance is a central immunologic mechanism which is responsible for allergy. Recently, desensitization for house dust mites was achieved successfully using interferon-gamma. Food-specific oral tolerance induction using IFN-γ for milk allergy of atopic dermatitis was tried in this study. A total of 250 atopic dermatitis patients participated in this study. Among these subjects, 85 patients were proved to have obvious milk allergy that were confirmed by double-blind placebo-controlled food challenge test enrolled in this study. Sixty-one (61) was treated according to Noh's protocol (N group). These patients received IFN-γ at a dose of 3x10⁶ IU for consecutive 7 day. They took food within 30 minutes after IFN-γ injection with a daily increasing quantity of 30 to 180 ml for 7 days. Seven patients were treated only by IFN-γ (G group). Nine patients were treated by only milk ingestion (M group). Remaining eight patients were control group who received placebo only instead of milk (C group). All of N group showed tolerance to milk without any adverse reaction to milk ingestion just after treatment. None of other three groups escape allergic reaction to milk even after treatment.

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Mechanisms of hemorrhage (HEM) induced neutrophil priming for acute lung injury (ALI): A role for MIP-2.

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Dysregulation of neutrophil (PMN) activation or priming is thought to be associated with ALI. Studies suggest that retention of functional PMNs in the lungs of trauma/sepsis patients is mediated by the suppression of apoptosis (Ao) by proinflammatory cytokines/chemokines. The human chemokine IL-8 is known to alter PMN chemotaxis and Ao. Preliminary ex vivo studies with the mouse homologue of IL-8, MIP-2, show a similar decrease in TNFα

induced Ao. Treatment with MIP-2 antibody led to an increase in Ao compared to the untreated mouse PMNs. We hypothesized that in vivo neutralization of MIP-2 following HEM would restore normal Ao function, thus decreasing priming for lung injury. To investigate this, mice were subjected to hemorrhagic shock (priming stimulus) followed at 24h by sepsis (cecal ligation and puncture [CLP])(triggering). Antibody to MIP-2 or control IgG was administered immediately following HEM 24h prior to CLP. Lung tissue myeloperoxidase (MPO), IL-6 and MIP-2 were measured and histology compared. Results showed that antibody neutralization of MIP-2 produced a suppression of lung MPO (PMN infiltration); a significant decrease in IL-6 ($p < 0.05$ vs Hem/CLP-IgG) and a decrease of MIP-2 in lung homogenates. Lung histology showed a decrease in congestion in the MIP-2 antibody treated group. These data suggest that MIP-2 plays a role in the suppression of mouse PMN Ao, and that as with the human IL-8, chemokine induced alterations in PMN Ao and/or chemotaxis contribute to the pathogenesis of ALI subsequent to shock and sepsis.

IMMUNE DEFICIENCIES (282)

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IN VIVO NK CELLS ACTIVATION DURING BACTERIAL INFECTION IN A PATIENT WITH SEVERE COMBINED IMMUNODEFICIENCY (SCID).

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Severe Combined Immunodeficiency (SCID) is a syndrome characterized by a deficit of functional T and B lymphocytes and constitute a model which enables to study the regulation of NK cells function in the absence of T and B cells. This report presents the phenotypic and functional characterization of the NK cells population from a 2 month old male patient with SCID during and after bacterial infection by *Staphylococcus Aureus*. Phenotypical analysis of peripheral blood cells, by flow cytometry with specific monoclonal antibodies, shows the absence of CD3+/CD4+ or CD3+/CD8+ cells (mature T lymphocytes), the absence of CD19+/CD21+ or CD19+/CD20+ cells (mature B lymphocytes) and the presence of a lymphoid population CD56+, CD16+, CD2+, CD7+, CD3 ϵ - phenotypically defined as NK cells. Results by double fluorescence of enriched NK cells show the expression, on CD56+ cells, of CD25 (interleukin-2 receptor α -chain) and HLA-DR antigens indicating the *in vivo* activation of NK cells during the *Staphylococcus Aureus* infection. A second phenotypical analysis made 14 days later, when the patient was asymptomatic and the hemoculture was negative, shows a significant decrease in the percentage of NK cells expressing HLA-DR antigens and a slight reduction in the percentage of NK cells expressing CD25 molecule. NK cells from SCID patient present increased cytotoxic activity against K562 target cells, before and after stimulation with interleukin-2 (IL-2), when compared with controls. We do not found significant differences in the cytotoxic activity of NK cells, prior and after IL-2 stimulation, between controls and the SCID patient 14 days post *Staphylococcus Aureus* infection. The fact that NK cells, from a patient with absolute absence of T and B lymphocytes, respond to bacterial infection in terms of activation and cytotoxicity could indicate either self regulation of NK cells activity in SCID or a regulation by other cells of non lymphoid lineage.

AUTOIMMUNE STATES (283-286)

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Possible roles of aberrant expression of B lymphocyte chemoattractant (BLC/CXCL13) in breakdown of immunological tolerance and autoantibody production in murine lupus

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B lymphocyte chemoattractant (BLC/CXCL13) expression was markedly enhanced in the thymus and kidney in aged BWF1 mice developing lupus nephritis, but not in similarly aged NZB and NZW mice. BLC positive cells were present in the cellular infiltrates in the target organs with a reticular pattern of staining. CD11b+CD11c+ myeloid dendritic cells were increased in the thymus and spleen in aged BWF1 mice and identified as the major cell source for BLC. CD4+ and CD8+ T cells as well as B cells were markedly increased in the thymus in aged BWF1 mice while CD4+CD8+ T cells were dramatically decreased. This dramatic change in the thymus of aged BWF1 mice was not observed in similarly aged NZB and NZW mice. B1 cells as well as B2 cells were increased in aged thymus and B1/B2 ratio in the thymus was also significantly higher than those in the spleen and peripheral blood in aged BWF1 mice. Interestingly, BLC showed preferential chemotactic activity for B1 cells derived from several mouse strains including non-autoimmune mice. Cell surface CXCR5 expression on B1 cells was significantly higher than that on B2 cells. Thus, aberrant high expression of BLC by myeloid mature DCs in aged BWF1 mice may play a pivotal role in breaking central tolerance in the thymus and in recruiting autoantibody-producing B cells in the development of murine lupus.

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IL-18 and IL-18BP have differing effects on the induction or recovery phase of the DSS model of colitis.

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IL-18 is an important signal in the development of the Th1 immune response which has been shown to be upregulated in intestinal biopsies from Crohn's disease patients. IL-18BP is a naturally occurring non-receptor molecule which neutralizes IL-18 activity. This study examined the effect of exogenously administered IL-18 and IL-18BP on the development of the DSS model of colitis in mice. Two separate experimental regimens were conducted. Firstly (induction regimen) colitis was induced in female 10-12 week old SJL/L mice by the ad libitum administration of 4% DSS (Mr 40 KD) in the drinking water from day 0 until necropsy on day 8. Rat IL-18 (10 micrograms per mouse) or human IL-18BP conjugated to human IgG1 Fc (IL-18BP-Fc, 5 mg/kg) were given every other day starting from day 0. Secondly, (recovery regimen) colitis was induced with DSS as above from day 0 to day 7, whereupon DSS was withdrawn and replaced with ordinary drinking water until necropsy on day 15. In this regimen, dosing with IL-18 and IL-18BP-Fc was started on day 4 and continued as above. In the induction regimen, IL-18 worsened large intestine histopathology, whereas IL-18BP-Fc substantially improved it. IL-18BP-Fc also improved disease-associated hematological parameters such as WBC and RBC counts, while IL-18 had no effect on those. Neither IL-18 nor IL-18bp-Fc had discernible effects on survival or weight loss. In the recovery regimen, IL-18 had no significant effect on large intestine histopathology, whereas IL-18BP-Fc significantly worsened the inflammation of the colon. IL-18 improved WBC and RBC counts, whereas IL-18BP-Fc significantly worsened them. Again, neither IL-18 nor IL-18BP-Fc had effects on survival or weight loss. These data suggest that there is a complex and dynamic interplay between IL-18 and IL-18BP in this model of colitis, depending on the phase of disease.

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CYTOKINE STIMULATION BY DEVELOPMENTAL ANTIGENS, A POTENTIAL MECHANISM FOR AUTOIMMUNE DISEASE.

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The MAGE (melanoma antigen) family genes are important in development and are expressed during embryogenesis. MAGE proteins are not expressed in

normal cells, except in spermatogonia. MAGE-A, -B, and -C genes code for proteins that are expressed in melanomas and other tumors. Using sera from two patients with systemic lupus erythematosus (SLE) we previously identified a member of the MAGE-B family, MAGE Xp-2, from a cDNA expression library from a HEP-2 laryngeal carcinoma cell line. This suggested that this gene is not only expressed in certain tumors, but also in autoimmune diseases. In this study we demonstrate the expression of MAGE-A1 in cells from synovial fluid aspirates from patients with juvenile rheumatoid arthritis (JRA) using RT-PCR. Monoclonal antibody to MAGE-A1 (Ab-4, Neomarkers, Fremont, CA) stains small and large aggregates of cells from synovial fluid. Flow cytometry shows expression of MAGE-1 in both the cytoplasm and on the cell surface. In vitro stimulation of peripheral blood mononuclear cells (PBMC) from patients with autoimmune diseases (e.g. Type 1 diabetes) with recombinant MAGE Xp-2 protein results in the release of pro-inflammatory cytokines (IL-1, IFN-gamma). We hypothesize that viral infections or other environmental triggers induce the expression or presentation of MAGE proteins, normally only expressed early in development. These antigens may not be tolerated as self antigens and may elicit an immune response (TH1 type) that initiates and perpetuates an autoimmune process.

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Suppression of autoimmune arthritis in IL-1-deficient mice in which T cell activation is impaired due to low levels of CD40L and OX40 expression on T cells

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Objective. To elucidate roles of Interleukin-1 (IL-1) in the development of two etiologically different Rheumatoid arthritis (RA) models; type II collagen (IIC)-induced arthritis (CIA) model and human T cell leukemia virus type I (HTLV-I) transgenic (Tg) mouse model. **Methods.** @For CIA model, DBA/1J background-IL-1 α ^{-/-}, -IL-1 β ^{-/-}, -IL-1 α β ^{-/-} and wild type littermate mice were immunized with IIC. For HTLV-I-Tg model, Balb/c background IL-1 β ^{-/-} or IL-1 α β ^{-/-} mice were crossed with HTLV-I-Tg mice. The effects of IL-1 deficiency were assessed by the following parameters. Development of arthritis was judged macroscopically and also microscopically. Serum antibody titer was measure by ELISA assay. Proliferative response of lymph node (LN) cells was assayed by [³H]-thymidine incorporation. Expression of T cell surface molecule CD40 ligand (L) and OX40 was determined by multi-color flow cytometric analysis. **Results.** The development of arthritis was markedly suppressed in IL-1 α β ^{-/-} mice in both models, although the effect was less prominent in HTLV-I-Tg. Deficiency of either IL-1 α or IL-1 β was enough to suppress the disease. Antibody production after immunization with IIC was normal in IL-1 α β ^{-/-} mice, while autoantibody production was suppressed in IL-1 α β ^{-/-} HTLV-I-Tg mice. In IL-1 α β ^{-/-} mice, T cell proliferating response against IIC was greatly reduced in both CIA and HTLV-I models, suggesting inefficiency of T cell activation. Furthermore, we found that expression of CD40L and OX40 on T cells was greatly reduced in IL-1 α β ^{-/-} mice. **Conclusion.** These observations suggest that T cell activation by IL-1 is important for the development of autoimmunity and arthritis in these mice.

IMMUNOMODULATORS/ IMMUNOREGULATION (287-296)

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Regulation of IFN-gamma gene expression in mouse peritoneal macrophage by cytokines.

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We previously reported that resting murine peritoneal macrophages (PM) express IL-12 receptors and biologically respond to IL-12 by secreting IFN-gamma. Moreover, we showed that IL-12 response of PM was regulated

differently by IFN-alpha and IL-18. The opposite effects exerted by these cytokines mirrored their mutual capacity of regulating in a negative or positive manner, respectively, the expression of IL-12R beta1 chain. To further characterize the mechanisms/factors involved in the biological response of PM to IL-12, we analyzed the effect of IL-15, a recently described macrophage-derived cytokine early produced in innate immunity responses, on the IL-12-stimulated production of IFN-gamma. Although IL-15 per se did not induce IFN-gamma production in PM, low doses of this cytokine markedly increased IFN-gamma production in IL-12-treated PM cultures with respect to PM treated with IL-12 alone. IL-15 resembles IL-2 in many of its biological activities. Moreover, IL-15 and IL-2 use shared receptor components for signal transduction. Thus, we investigated whether IL-2 exhibited some modulatory effect on the biological response of PM to IL-12. In contrast to IL-15, treatment of PM with IL-2 was sufficient to directly induce IFN-gamma production which was further increased by the simultaneous addition of IL-12. Several reports have demonstrated that the macrophage population resident in the peritoneum of mice is extremely heterogeneous in terms of phenotype and function. Preliminary experiments suggest that specific macrophage subpopulations might differ for their capacity of producing IFN-gamma in response to different stimuli. Studies are in progress to phenotypically characterize the IFN-gamma-producing cells under different experimental conditions. The finding that macrophages respond to IL-12 and that this response can be differently regulated by a number of cytokines produced early in the course of innate immunity response further highlights the important role of these cells in linking innate and adaptive immunity.

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Engagement of the phosphatidylserine receptor on dendritic cells inhibits their maturation by a TGFbeta-independent mechanism.

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The phosphatidylserine receptor (PSR) has been shown to be present on the surface of many different phagocytic cell types and to play a key role in the uptake of apoptotic cells. We investigated whether the PSR was present on human dendritic cells and examined some of the effects of its engagement on dendritic cells. Engagement of the PSR on dendritic cells appears to result in upregulation of its own surface expression. Also, PSR ligation followed by stimulation with a pro-inflammatory stimuli inhibited differentiation of immature dendritic cells into mature antigen presenting cells as determined by the surface expression of CD83. Although engagement of PSR on the surface of dendritic cells resulted in TGFbeta secretion, TGFbeta did not appear to be acting in an autocrine/paracrine manner to produce the inhibitory effects on maturation. Thus, the PSR may prevent inappropriate immune responses to self by inhibiting the maturation of dendritic cells that have captured self-antigens through phagocytosis of apoptotic cells and this process appears to be independent of TGFbeta.

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INTERLEUKIN-1 STIMULATES WOUND HEALING AND LOCAL IMMUNITY IN PATIENTS WITH SKIN TROPHIC ULCERS

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Interleukin-1 (IL-1) is one of the most important cytokines that stimulates immune and inflammatory responses. IL-1 has pleiotropic mode of biological activity and also activates connective tissue metabolism and regeneration of traumatic lesions. The aim of this study was to investigate the possibility of local IL-1 application for the therapy of skin trophic ulcers in patients with diabetes and to study mechanisms of local IL-1-induced wound healing and immunostimulation. Recombinant human IL-1 beta has been used in a form ointment to treat patients with chronic skin ulcers after failure of routine therapy. IL-1 ointment was applied directly to the lesion site once per day for 5-10 days. This IL-1 local treatment led to a clinical improvement in majority of patients. In all cases in comparison with traditional methods of therapy IL-caused more rapid development of granulations, epithelization and more quick

decrease in the lesion size. The most profound increase in wound healing was observed especially in infected lesions. Absolutely no systemic side effects were observed during IL-1 local therapy. Cytological data showed that IL-1 induced changes in the numbers of various types of leukocytes and increased the numbers of fibroblasts in wound cell smears. IL-1 application caused an increase in initially depressed migration to fMLP, superoxide production, adhesion to plastic and phagocytosis of leukocytes isolated from the inflammatory sites. Obtained results indicate that IL-1 can be used with high efficiency for local therapy without complications that frequently take place during systemic cytokine treatment.

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The soluble form of the IL-1 Receptor accessory protein (sIL-1RAcP) induces a bi-phasic response upon IL-1 stimulation.

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To assess the role of the naturally occurring soluble form of IL-1RAcP (sIL-1RAcP) in IL-1 signalling, the effect of sIL-1RAcP was evaluated on IL-1 induced Nitric Oxide (NO) production by SV40 immortalised chondrocyte cell-line (H1 cells) in-vitro, using two different approaches. First, H1 cells were transfected with an adenoviral vector containing the natural mRNA splice variant of murine sIL-1RAcP (Ad5 sIL-1RAcP RGD). Secondly, H1 cells were co-cultured in transwell systems with a stable transfected 3T3 NIH fibroblast cell line expressing sIL-1RAcP. Addition of sIL-1RAcP to H1-chondrocytes by adenoviral transfection stimulated the NO production by H1 cells after addition of IL-1 α , whereas high amounts of sIL-1RAcP produced in co-culture by transfected 3T3 NIH fibroblasts inhibited IL-1 induced NO production by H1 cells for up to 34%. To assess the effect of sIL-1RAcP on IL-1 mediated arthritis in-vivo, male DBA/1 mice were immunised against bovine collagen type II followed by intra-articular injection of Ad5 sIL-1RAcP RGD or 2*10⁵ stable transfected sIL-1RAcP producing 3T3 NIH fibroblasts before onset of collagen induced arthritis (CIA). Adenoviral transfection of the sIL-1RAcP gene increased incidence of CIA compared to the luciferase or IL-1RA gene, whereas injection of high producing sIL-1RAcP 3T3 NIH fibroblasts impeded the disease incidence. These results reveal a bi-phasic effect in-vivo and in-vitro of sIL-1RAcP upon IL-1 α mediated signaling, which strongly suggests sIL-1RAcP concentration dependent modulation of IL-1 receptor complex formation.

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EXPRESSION OF FUNCTIONAL P2z/P2X7 PURINERGIC RECEPTOR BY RAT ALVEOLAR MACROPHAGES AND ITS MODULATION BY CYTOKINES

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Extracellular ATP (ATPe) mediates a variety of effects on immune cells mainly through activation of the P2z/P2X7 nucleotide receptor. This purinergic receptor has been described in murine macrophage lines, peritoneal macrophages, human monocytes/macrophages and monocytic cell lines. As yet, there is lack of evidence for its expression by resident alveolar macrophages (AM) whether at the functional, pharmacologic or molecular level. In this study, we investigated the effects of ATPe on rat AM obtained by bronchoalveolar lavage. AM were susceptible to ATP-mediated permeabilization as evaluated by uptake of low-molecular-weight fluorescent probes such as ethidium bromide, YO-PRO1 and lucifer yellow (42% in ATP-treated cells compared to 2% in controls). ATPe was active in the millimolar range (3-5mM) and its effect was blocked by oxidized ATP (100-300uM), an irreversible P2z/p2X7 blocker. Dye uptake was inhibited by extracellular calcium, magnesium and zinc, as typically defined for P2X7 receptor. Pre-incubation with IFN- γ (100-1000U/ml) and IFN+TNF (10ng/ml) for 24 hr significantly enhanced ATP-

mediated permeabilization. ATPe also induced a significant increase in apoptotic AM (30%) compared to untreated cells (0.5%) as evidenced by increased nuclear fragmentation following Hoechst staining, increased oligonucleosomes in cytoplasmic fractions of AM and DNA ladder formation. In addition, ATPe (5mM) induced significant release of IL-1- α from LPS-primed AM. Furthermore, immunocytochemical analysis using anti-P2X7 Ab revealed that 30-40% of AM expressed P2X7 and that immunoreactivity was particularly apparent at junctions of fused cells during MCSF- and GMCSF-induced MGC formation. Our observations demonstrate that alveolar macrophages exhibit all the characteristic features of a functional P2X7 receptor.

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ASF as a Novel Immunoregulator

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Inflammatory responses in the central nervous system are tightly regulated. A key cell type involved in such responses is the microglia; a resident bone marrow derived population whose function is to present antigen in the CNS. In order to define novel molecules on microglia that regulate inflammation, we have generated a panel of monoclonal antibodies directed against microglial cells. One such antibody, clone name TLD-1A8A, appears to have immunomodulatory properties both in vitro and in vivo. We have determined that TLD-1A8A is directed against a protein called antiseptory factor, ASF. ASF has previously been identified by another group as a protein which inhibits intestinal fluid secretion induced by cholera toxin. Our data show that antiseptory factor also has immunological functions. Administration of TLD-1A8A during the course of experimental autoimmune encephalomyelitis results in increased severity and duration of disease. Administration of the antibody in T cell proliferation assays and mixed lymphocyte response assays results in increased proliferation of T cells. We show here that ASF is produced by macrophages, and its expression is affected by inflammatory stimuli in vivo. The data thus far are consistent with the hypothesis that antiseptory factor is a novel molecule produced by cells of the macrophage lineage which has modulatory effects on T cells. Future studies include determining what these modulatory effects may be by further characterizing the biological and immunological effects of antiseptory factor. These studies will potentially lead to an increased understanding of the immune response both in the CNS and in the periphery.

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Oral ascorbic acid and beta-glucan affect cytokine expression differently after an LPS challenge.

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β -glucan from *Saccharomyces cerevisiae* and ascorbic acid both can be immunomodulators of innate immune cells and when given together, weight gains of some species were enhanced. Our current objective was to investigate acute cytokine responses after a lipopolysaccharide (LPS) challenge in swine, given an oral β -glucan product and/or an ascorbic acid product. Pigs (n=8/trt) received carrier only (Control), a β -glucan product given as 2.5% of the diet (BG), an ascorbic acid product delivered at 75 ppm (AA), or both (Both) from birth until 28 days of-age, then LPS was delivered i.v. (150 ug/kg). Three hours after the LPS challenge, pigs were euthanized, a lung lavage was used to obtain pulmonary alveolar macrophages (PAMs), and lung, spleen, and liver tissues collected and total RNA was extracted for semi-quantitative RT-PCR. Interleukin-1 (IL-1) expression in PAMs was greater for pigs receiving BG than for the Both and Control pigs ($P < 0.05$) and tended ($P=0.06$) to be greater than that of AA supplemented pigs. AA pigs had the least IL-1 expression in spleens ($P < 0.05$) compared to BG and Both treatments and tended to be less ($P=0.06$) than IL-1 expression in the spleens of Control pigs. BG also increased IL-1 expression in the liver compared to the AA supplemented pigs ($P < 0.05$). IL-1 receptor antagonist (Ra) expression tended to increase with BG compared to Controls in PAMs and liver expression of IL-1Ra of pigs receiving Both

tended to be greater than AA treated pigs ($P < 0.10$). TNF- α expression in lung tissue tended ($P < 0.10$) to be greatest for BG and Both pigs compared to AA and Control pigs. TNF- α expression was not detectable in PAMs, spleen cells, or liver cells. These data show that oral BG results in the greatest and oral AA supplementation results in the least RNA cytokine expression 3 hr after an LPS challenge.

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Effects of Androstenediol on Microscopic Lesions of Ulcerative Colitis in a Rat Model of Inflammatory Bowel Disease

B. Richard, C. Meschter*, C. Reading, U. Orlinska, C. Ahlem. Hollis-Eden Pharmaceuticals, San Diego CA, *Comparative Biosciences, Santa Clara, CA. Androstenediol (AET) is a potent immunomodulating and anti-inflammatory drug which may be beneficial in inflammatory bowel diseases (IBD). AET counteracts stress-induced immune suppression by modifying Th1/Th2 inflammatory cytokines. The purpose of this study was to evaluate the amelioration of clinical signs in relation to histopathological changes of the large intestines from rats treated with AET and Sulfasalazine (SZ) in a model of ulcerative colitis. Distal colitis was induced by colonic instillation of 2,4-dinitrobenzenesulfonic acid (DNBS). AET or vehicle was administered at 6 daily doses by subcutaneous injection of 10 mg/kg, first dose being given 30 min. after DNBS challenge. SZ was administered at 7 daily oral doses of 300 mg/kg, first two doses given 24 hours and 2 hours before DNBS challenge. Animals were observed daily for 1 week or two weeks. The intestines were examined macroscopically and microscopically. AET reduced incidence of diarrhea by 40-50% compared to 32% with SZ. AET was more effective than SZ in reducing the occurrence and severity of ulcers and associated inflammatory reactions. The ulcers were smaller and there was more preservation of the mucosa. Only 2/10 animals treated with AET had severe lesions and 4/10 animals had no ulcers compared to vehicle group in which severe ulcers were predominant (8/10). Only 4/10 animals had severe inflammation compared to 8/10 animals in the vehicle group. In conclusion, AET was more effective than SZ and improved histopathological features.

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Administration of β -AET to burned mice modifies bone loss, structure and dynamics

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β -Androstenediol (β -AET) possesses anti-inflammatory and immunoregulatory properties in vitro and in vivo. We have shown an improvement of inflammatory bowel disease symptoms in rats. In this study, we investigated the effect of β -AET on bone loss, structure and dynamics in thermally-injured mice, a model of trauma and immune changes. Bone loss in burned mice is attributed to both inflammation and glucocorticoids. Mice received 20% total body surface area burn. β -AET was injected subcutaneously at 0.3, 0.6 and 3.0mg/mouse immediately after the thermal injury, and a second dose was given 48 hours later. The administration was continued 3x/week for 4 weeks. We observed partial protection against loss of bone mineral content with two lower β -AET doses measured by x-ray absorptiometry. With 0.6mg/mouse wet and dry femur weights were significantly ($p < 0.01$) greater than in vehicle-treated burned mice. Likewise, the femur ash weight was significantly ($p < 0.01$) greater than in vehicle-treated burned mice. Histomorphometry of the cortical bone suggest an increase in the bone formation rates in the low or medium doses with a maintenance of beneficial cortical bone structure. Our data suggest that β -AET at the low and medium doses have bone sparing effects in this model of thermal injury induced bone loss. Our data support the hypothesis that modulation of the immune system may have beneficial actions on skeletal tissues. We speculate that in burn trauma β -AET functions as an anti-inflammatory and anti-glucocorticoid steroid.

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Increased IL-10 and decreased IL-12 production correlate with reduced antigen presentation in alcohol-exposed myeloid dendritic cells

Pranoti Mandrekar, Donna Catalano and Gyongyi Szabo. University of Massachusetts Medical Center, Worcester, MA. Antigen-specific T cell proliferation and DTH responses are affected by acute and chronic alcohol exposure of monocytes in-vitro as well as in-vivo. Maturation of monocytes to specialized antigen-presenting dendritic cells is regulated by cytokine-mediated events. This process may therefore be accelerated or inhibited by various chemical agents or drugs. Here, we hypothesize that alcohol exposure in-vitro of human peripheral blood monocytes may affect their maturation to dendritic cells (DCs) and DC accessory cell function leading to immunosuppression. DCs were generated in-vitro from normal human blood monocytes with GM-CSF (100U/ml) plus IL-4 (200U/ml) in the presence or absence of physiologically relevant doses of alcohol (25mM) for 7 days. DC cell viability and apoptosis was not affected by alcohol as determined using annexin and propidium iodide on day 7. DCs were characterized by their increased expression of HLA-DR, CD1a, CD86, CD80, CD40 and decreased expression of CD14 as compared to monocytes. Our results indicate that accessory cell function of DCs generated in the presence of alcohol was decreased ($45 \pm 11\%$) as determined in a mixed lymphocyte reaction. We also observed decreased tetanus-toxoid induced T cell proliferation ($42 \pm 16\%$) in the presence of alcohol-exposed DCs. Interestingly, DCs generated in the presence of alcohol showed decreased IL-12 production and increased IL-10 production. Autocrine IL-10 produced by DCs has been shown to prevent DC maturation in-vitro and also have a inhibitory effect on IL-12 production. Our results here suggest that increased IL-10 production of alcohol-exposed DCs may impair their maturation and hence attribute to their decreased antigen presenting function.

IMMUNOPHARMACOLOGY (297-298)

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Apoptosis-inducing activity of novel triterpene saponins, securiosides A and B against M-CSF-stimulated macrophages

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It is widely recognized that macrophages in peripheral tissues often proliferate under pathological conditions such as tumors, inflammation and atherosclerosis. Because macrophage accumulation is believed to be a factor regulating the pathological process of the diseases, substances that regulate macrophage growth or survival may be useful for disease control. To identify a substance that inhibits macrophage proliferation, we performed a screening study among hot-water extracts of plant materials that have been traditionally used as Chinese medicines and are known to have anti-inflammatory activity. As the result, we found that two novel triterpene saponins (named securiosides A and B) from roots of *Securidaca inappendiculata* induced apoptotic cell death of murine peritoneal macrophages. The cytotoxic activity seemed to be specific to peripheral macrophages, since it showed a weak effect on several tumor cell lines including a macrophage-like cell line, J-774.1, normal fibroblasts, lymphocytes, and bone marrow cells. Importantly, securiosides induced macrophage apoptosis only when the cells were stimulated by M-CSF/CSF-1, whereas the saponins did not potentially affect the viability of macrophages cultured without M-CSF or with granulocyte/macrophage-CSF. Securioside B induced activation of caspase 3 and caspase 9, but not caspase 8 in M-CSF-stimulated macrophages. It also induced cytochrome c translocation from mitochondria to the cytosol, suggesting it induces macrophage apoptosis through mitochondria-dependent pathway. In conclusion, securiosides A and B may be the primary compounds of new drugs for the treatment of pathological states in which M-CSF-stimulated macrophage proliferation occurs.

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Inhibition of Macrophage Migration Inhibitory Factor (MIF) Tautomerase and Biological Activity by the Acetaminophen Metabolite, NAPQI.

Peter D. Senter*, Yousef Al-Abed, Christine N. Metz, Fabio Benigni, Robert A. Mitchell, J. Chesney, Jianlin Han, Carlos G. Gartner+, Sidney D. Nelson+, Richard Bucala. *Cytokine PharmaSciences Inc., Seattle, WA, 98119; The Picower Institute for Medical Research, New York, 11030; and the +Department of Medicinal Chemistry, University of Washington, Seattle, WA, 98195. Macrophage migration inhibitory factor (MIF) has emerged to be an important regulator of the innate and acquired immune response and is critically involved in the development of septic shock, arthritis, and glomerulonephritis. While the biological activities of MIF are presumed to require a receptor-based mechanism of action, the protein is also a tauto-merase and has a catalytically active N-terminal proline that is invariant in the structurally homologous bacterial isomerases, -4-oxalo-crotonate tautomerase, and 5-carboxymethyl-2-hydroxy-muconate isomerase. This observation raises the possibility that MIF may exert its biological action via an enzymatic reaction. Nevertheless, physiologically relevant substrates for MIF have not been identified, nor have site-directed mutagenesis studies consistently supported the requirement for a functional catalytic site. To provide insight into the role of the MIF catalytic activity as well as provide an approach to the design of new anti-inflammatory agents, we have explored the capacity of a class of quinone compounds related to the model MIF substrate, dopachrome, to inhibit the isomerase and the biological functions of MIF. We report that the iminoquinone metabolite of acetaminophen, N-acetyl-p-benzo-quinone imine (NAPQI), inhibits both the isomerase and the biological activities of MIF by covalent reaction with the active site of the protein. These results point to a powerful approach for the design of small molecule inhibitors of MIF based on interaction with its catalytic site, and constitute the first example of a pharmacophore capable of irreversibly inhibiting the action of a pro-inflammatory cytokine.

IMMUNOTHERAPY (299-307)

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ANTI-HUMAN INTERFERON-GAMMA ANTIBODIES IN THE TREATMENT OF PATIENTS WITH CORNEAL TRANSPLANT REJECTION

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[Objective] To determine the efficacy of the use of F(ab)2 fragments of anti-human interferon-gamma antibodies (AB) in the treatment of corneal transplant rejection after penetrating keratoplasty. [Method] AB obtained by goat immunization were used for instillations into the eyes. 13 patients experiencing transplant rejection at different periods (3 months to 13 years) after penetrating keratoplasty were enrolled in the study. In all patients the corneal transplant was half-transparent, edematous, and cloudy. Vision was limited to distinguishing hand movement in front of the eyes. Standard treatment of transplant rejection with steroids, antibiotics, anti-inflammatory drugs, and vitamins did not produce any improvement. Standard treatment was stopped, and AB were given at 2-3 drops three times a day for 7-10 days. Patients were observed for 3 months. [Results] Two to three days after start of treatment, transplant transparency improved, edema dropped. Visual acuity increased. At the end of the first week, the transplant became almost fully transparent and inflammation of the eye disappeared. Visual acuity increased to 0.2-0.3. This condition was maintained through the end of the treatment. Observation continues. [Conclusion] Anticytokine therapy (AB) can be considered a promising, safe, and effective approach to halting transplant rejection of the cornea after penetrating keratoplasty. Rare second rejections can be treated with the same agent. AB may also be used for the prevention of transplant rejection. These results support our hypothesis (S.Skurkovich, et al., Nature, 1974, vol. 247) that anticytokine therapy may be used for the treatment of autoimmune processes that occur in the transplant rejection reaction.

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Tumoricidal Activation of Macrophages and Effector Cells by Liposome-encapsulated (MLV) CGP31362 plus IL-2: Rapid Release of Interferon Gamma and Inflammatory Cytokines.

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The growth of spontaneous lung metastasis of murine renal adenocarcinoma (RENCA) implanted into the kidney is inhibited by systemic administration of the synthetic macrophage activator, MLV31362 plus IL-2. The number of individual lung tumor nodules was reduced from a median of 32 (range 16-63) in control mice to 12 (7-127) in the lungs of mice treated with this form of therapy. IL-2 encapsulated into the liposomes resulted in a 5-fold increase of IL-2 deposition into the lungs and RES tissue compared to free-form IL-2. This therapy resulted in tumoricidal activation of alveolar macrophages (> 40% killing of RENCA target cells) and increased NK activity (> 45% kill of YAK lymphoma). In addition, strong LAK cell killing was induced by incubation of cells with the MLV31362 + IL-2 (> 70% kill of RENCA). Cytokine analysis by ELISPOT assay revealed that incubation of spleen cells with MLV31362 + IL-2 resulted in the rapid release of IFN-gamma, IL-6, IL-10 and TNF. The constitutive release of IL-6, IL-10 and TNF by macrophages was enhanced by activation. The fast release of IFN-gamma was limited to the first 2 hours although NFkappaB and mRNA for IFN-gamma were upregulated. Mixtures of cell types and their cytokines within the tumor microenvironment modulate features of effector cell response to tumor.

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Construction of MAFJ6-1 DNA Vaccine for Tumors Immunotherapy

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Tumor DNA vaccination, targeting the tumor associated antigen (TAA), is an attractive strategy for the immunotherapy of tumors. MAFJ6-1, macrophage colony stimulating factor (M-CSF) like membrane associated factor, was found from human leukemic cell line J6-1 (Wu, et al. Leukemia Res 1994,18:843) The aberrant expression of M-CSF is associated with the pathogenesis of several solid tumors, as well as hematopoietic malignancies. Therefore, MAFJ6-1 might be a potential target for the immunotherapy of tumors. To elucidate the possible application of MAFJ6-1 DNA vaccine, the cDNA fragment of MAFJ6-1 was cloned from J6-1 cells using RT-PCR with the M-CSF specific forward primers (5'-GAG CCA GCT GCC CCG TAT GAC-3') and reverse primers (5'-CCC TCT ACA CTG GCA GTT CCA C-3'). The cDNA sequences showed a 768bp open reading frame (ORF) encoding the extracellular domain and transmembrane segment with 99.2% homology to m-M-CSF. Interestingly, 6 site mutations including 2 synonymous mutations and 4 missense mutations were detected by Blast software. This cDNA fragment was inserted into a mammalian expression plasmid pTARGET (Promega) and transfected into Cos-7 cells via LIPOFECTAMINE (GIBCO BRL). The expression of recombinant plasmid was demonstrated by ABC immunocytochemistry using monoclonal antibody specific for M-CSF. To construct MAFJ6-1 DNA vaccine, then we subcloned the cDNA fragment into plasmid pcDNA3.1, which, designated as pcDNA3.1-M, was confirmed by restriction endonuclease analysis. To understand the immune response induced by this DNA vaccine in BALB/C (H-2d) mice, a tumor model needed to be established following inoculation of syngeneic sp2/0 cells stably transfected with pcDNA3.1-M. After 20th passage, one stably transfected sp2/0 cell line was established. In conclusion, we constructed MAFJ6-1 DNA vaccine and established stably transfected sp2/0 cells serving as the target cells for MAFJ6-1 DNA vaccine. However, the protective immunity generated by this DNA vaccine remains to be evaluated in further work.

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Correction of genetically determined low and non responsiveness to Hepatitis B surface antigen by vaccination with a mixture of alum-adsorbed HBsAg and alum-adsorbed cytokines.

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Lack of response to Hepatitis B vaccination remains a problem for individuals directly at risk of hepatitis B infection, e.g. those working in health care institutions. In mice, the level of the humoral immune response to HBsAg is regulated by MHC haplotype-linked immune response genes, as was convincingly shown by Milich et al. Whereas mice carrying the H-2d haplotype (e.g. BALB/c) respond with high anti-HBsAg antibody titers, H-2b mice (e.g. C57BL/6) are intermediate and H-2s mice (e.g. SJL) are non responders to HBsAg. We have recently shown that alum-adsorbed cytokines are potent adjuvants. By addition of alum-adsorbed IL-2 we were able to induce protective prophylactic as well as therapeutic curative cellular immune responses to weakly immunogenic tumor cells. Since in these formulations the cytokines were applied locally and in low doses no side effects were observed. We therefore assumed that the addition of alum-adsorbed cytokines to conventional Hepatitis B vaccines might help to induce immune responses in low or non responder mice. BALB/c high responder, C57BL/6 intermediate responder and SJL low responder mice were immunized with vaccines containing alum adsorbed HBsAg as the standard vaccine component and, in addition, alum-adsorbed IL-2 or GM-CSF as adjuvants. The results obtained clearly show a) that addition of alum adsorbed cytokines to the standard HBsAg-alum vaccine strongly and significantly enhances the humoral immune response to HBsAg in BALB/c responder and C57BL/6 intermediate responder mice and b) that the response in non responder SJL mice is also enhanced strongly and significantly, although not reaching the same level as in the high and intermediate responder mice. c) that vaccination with the IL-2-alum containing vaccines induced higher levels of IgG2a than vaccination with the conventional HBsAg-alum vaccines. These results indicate that the application of alum-adsorbed cytokines in addition to the standard HBsAg vaccine might help to overcome low or non responsiveness in humans as well.

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Tumor vaccines containing alum-adsorbed IL-2 as an adjuvant stimulate protective cellular immune responses.

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The murine melanoma B16F10 is an aggressively growing highly metastatic tumor. Prophylactic vaccination (i.e. vaccination prior to tumor induction) with irradiated tumor cells alone has no or only slight life-prolongating effect. Addition of alum-adsorbed IL-2 to the irradiated tumor cells significantly prolongs survival time of the vaccinated animals. In order to study the cellular events leading to the observed inhibition of tumor growth, we have investigated the composition of the cellular infiltrate at the inoculation site and in the lymph nodes draining the affected region. After vaccination with irradiated tumor cells alone the inoculation site was infiltrated by a marginal number of inflammatory cells. However, after vaccination with the IL-2-alum containing vaccine, the inflammation was much stronger and a cellular infiltrate was found in as well as around the inoculation site. In both cases, PKH-2 labeled tumor cells were degraded, the fragments were taken up by antigen presenting cells and carried to the regional lymph nodes where they were detected in Ia-expressing cells. Although addition of alum-adsorbed IL-2 induced a stronger local inflammatory infiltrate, the amount of tumor cell debris in the lymph nodes was approximately the same. Flowcytometric investigation of the cell populations in the lymph nodes 6 days after vaccination revealed that the expression of co-stimulatory molecules B7-2 and CD40 was significantly increased in the lymph nodes of mice vaccinated with the alum-adsorbed IL-2 containing vaccines. Ten days after vaccination, the lymph nodes of these mice were significantly enlarged and contained high fractions of B cells, CD4 cells, and CD8 cells. As a consequence, it can be

concluded that the protective immunity induced by vaccination with alum-adsorbed IL-2 is caused - by the increased inflammatory infiltration of the inoculum - by the increased expression costimulatory molecules in the affected lymph node, and - by activation and proliferation of effector cells in the lymph nodes.

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Anti-tumor Effects of Liposomal IL-2 in a MCA 38 Colon Liver Metastases Tumor Model.

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Liposomal IL-2, L[IL-2], differs from soluble IL-2 in pharmacokinetics and biodistribution. When injected intravenously, L[IL-2] distributes to the organs of the reticuloendothelial system, including the liver and spleen, while soluble IL-2 is quickly eliminated by the kidneys. These favorable characteristics were evaluated in a metastatic model of colon cancer. In this model, MCA 38 cells metastasize to the liver after intra-splenic injection. Four days following tumor cell inoculation, mice were treated with 5 daily IV injections of empty liposomes or L[IL-2] (total dose = 500000 IU). Eleven days after the last injection the mice were euthanized and the number of liver metastases, as well as the weight of splenic tumors, were determined. Dose, schedule and the effects of chemotherapy were tested. Results: Treatment of mice with intravenous L[IL-2] significantly inhibited the growth of MCA 38 tumors in the spleen and decreased the number of liver metastases when compared to mice receiving empty liposomes. The mechanism of inhibition will be discussed.

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The Kinetics of an IFN Gamma Response

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For effective anti-tumor immunity it is important to achieve a strong immune response, and to maintain that response for the length of time necessary to achieve tumor rejection. To determine the kinetics of an anti-tumor vaccine-elicited response, we measured IFN- γ production from vaccinated splenocytes immediately *ex-vivo*. We vaccinated BALB/c mice i.p. with a β -galactosidase carrying virus. At various times after vaccination, splenocytes from the vaccinated mice were harvested and stimulated immediately *ex-vivo* for three hours, and the mRNA was extracted and measured by real time PCR. IFN- γ mRNA, normalized to CD8 mRNA was assayed to estimate the strength of the immune response. We boosted mice at different time points after vaccination and estimated their IFN- γ levels to select the best boosting regimen. This work provides guidelines for establishing a boosting regimen to maintain high level of immunity after vaccination, in order to enhance anti-tumor immunity.

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OPTIMIZATION OF MOLECULAR ADJUVANTS FOR VACCINES: SP1017 COPOLYMER ENHANCES EXPRESSION OF CYTOKINE GENES IN MUSCLE

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Granulocyte-macrophage colony stimulating factor (GM-CSF) DNA demonstrates an adjuvant effect in conjunction with vaccines. It recruits and activates dendritic cells for enhanced presentation of tumor antigens. Activated dendritic cells produce cytokines and chemokines, recruiting other immune effector cells. However, low levels and short duration of GM-CSF expression limit the effectiveness of naked DNA delivery. To elevate and sustain production of GM-CSF at the site of vaccination, we examined the use of SP1017, a combination of non-ionic block copolymers (Lemieux, P. et al., Gene Therapy. 2000, 7, 986-991). SP1017 significantly enhances both the magnitude and duration of reporter gene expression in muscle. Moreover,

injection of SP1017 together with human melanoma antigen (TRP-2) DNA, prevents the growth of B16 melanoma tumors in C57BL/6 mice. To further optimize the use of molecular adjuvants, we have investigated SP1017 for intra-muscular delivery of GM-CSF DNA. Mice were injected with a mixture of SP1017 and GM-CSF plasmid DNA. Injected muscles were harvested at multiple time points and assayed for cytokine production. SP1017 promoted an earlier onset and a 10-fold increase in GM-CSF production when compared to GM-CSF DNA alone. As expected, increased GM-CSF production was associated with higher levels of MCP-1. Finally, the use of SP1017 lead to an increase in IL-12 production at 96 hours, suggesting improved recruitment of dendritic cells/macrophages. SP1017 plus empty vector DNA did not elicit production of cytokines or chemokines. These results indicate that SP1017 is a promising alternative to naked DNA. Its enhancement of the effects of molecular adjuvant has implications for the design of vaccine trials.

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IL-12 and IL-18 DNA: Molecular Adjuvants for Antitumor Vaccines

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Immunization with plasmid DNA is a method to induce potent antibody and T-cell responses to otherwise poorly immunogenic antigens. We have previously shown that immunization of mice with xenogeneic (human) gp100 DNA results in protection of 50-60% of C57BL/6 mice challenged with a syngeneic melanoma (B16) via a CD8+ T-cell dependent mechanism. In an attempt to improve immunogenicity, we have explored whether administration of hgp100 in combination with the genes for IL-12 and IL-18 can increase tumor protection and precursor T cell frequency. The addition of the genes for IL-12 and IL-18 resulted in improved protection from B16 challenge. Peptide-specific T cell responses were evaluated by measuring intracellular cytokines by 4-color flow cytometry (FACS). Combining hgp100 with either IL-12 or IL-12 and IL-18 DNA increased the percentage of activated CD8+ cells in draining lymph nodes of immunized mice that secrete IFN- γ in response to syngeneic mouse gp100. Interestingly IL-2 secretion by activated CD8+ T cells was also increased when hgp100 was combined with IL-12 and IL-18 DNA, suggesting the induction of a memory response. Molecular adjuvants such as IL-12 and IL-18 DNA can enhance the immune response against syngeneic B16 melanoma cells in mice treated with a suboptimal DNA immunization. In addition, the combination of the two cytokine genes may promote a more sustained response. These results have implications for the design of future clinical trials of anti-tumor vaccines.

DIAGNOSTICS/EXPERIMENTAL THERAPIES (308-313)

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Comparison of cytokine measurements by ELISA with cytokine mRNA estimations by quantitative RT-PCR: is there a correlation between the two?

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Cytokine secretion is measured in many laboratories to give an indication of the type of immune response elicited by a particular antigen or a microbial infection. Although there are many different techniques available, some laboratories may only use one method to assay cytokine type and quantity. However, since all assay systems have their disadvantages it is important to choose the correct techniques to provide an accurate account of what is really happening in any system that is under investigation. In this study the amounts of interferon gamma and interleukin 4 present in tissue culture supernatants have been quantified by ELISA and the amount of cytokine message present in the cells has been measured by quantitative RT-PCR. These measurements have been performed in time-course experiments to investigate whether there

is any correlation between the two techniques, and to what effect the choice of time points has on the results gained.

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COMBINATION OF LACTAM STEROIDS WITH INTERFERON-ALPHA FOR THE TREATMENT OF PANCREATIC ADENOCARCINOMA CELLS

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Pancreatic cancer is a devastating disease with poor prognosis. Current cytotoxic chemotherapy results in low response and short patient survival. On the other hand the therapeutic efficiency of non toxic therapy with cytokines or steroid hormones seems to be promising. We studied the growth inhibitory effect of two modified steroids, lactadron (an A ring lactam derivative of 3 beta, 5 alpha androstan, LA) and lactestron (an A ring lactam derivative of estrone, LE), as well as the inhibitory effect of interferon-alpha (INF-alpha) on three human pancreatic adenocarcinoma cell lines, Mia PaCa2, PC-02 and PC-08. The cell lines PC-02 and PC-08 were isolated from the ascitic fluid of two male patients with advanced pancreatic adenocarcinoma and were characterized morphologically, immunophenotypically and karyotypically (over 50 chromosomes). Both PC-02 and PC-08 cell lines were positive in the presence of androgen and estrogen receptors. The inhibitory effect of combinations between INF-alpha and lactam steroids (LE, LA) on growth of the three experimental cell lines was further tested. The cell growth was estimated with the MTT colorimetric assay and the compound concentrations that produced 50% inhibition of cell growth (IG50) in 72 hours of culture were calculated. The IG50s that were performed in Mia PaCa2, PC-02 and PC-08 cells, were for LA, 1,5 μ l, 0,9 μ l and 1,9 μ l respectively, for LE were over 5 μ l in all three cell lines tested, for INF-alpha were 3350, 2600 and 3100 iu/ml respectively. The effect of simultaneous treatment with LE or LA and INF-alpha was nearly cumulative. When cells were pretreated with 500 or 2000 iu/ml of INF-alpha for 24 hours the growth inhibition induced by LE was significantly ($p < 0,001$) increased (IG50: 0,9-1,3 μ l) but surprisingly the exhibited inhibition on cell growth by LA was almost completely diminished (IG50 over 5 μ l). On contrary, when cells were pretreated for 24 hours with 0,1 or 0,5 μ l of LA the IG50s of INF-alpha were significantly ($p < 0,01$) decreased (IG50: 1800-2200 iu/ml). The above results indicate the existence of important synergism between lactam steroids and INF-alpha which may be proven useful in prospective therapy of pancreatic cancer.

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Mouse Cytokine Multiplex Bead Immunoassay: Correlation with Traditional ELISAs

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Mouse model systems are commonly used to study Th1/Th2 and inflammatory responses. Despite their utility, mouse model systems suffer from the paucity of sample volume available to dissect the complex homeostasis involved in immune responsiveness. This drawback can be ameliorated by technologies that allow multiple analytes to be measured simultaneously in a single sample. Nevertheless, any technology offered must yield sensitivity, specificity and quantitation comparable to existing ELISA methods commonly employed. The aim of this study was to describe the BioSource International, Inc. Multiplex Bead Immunoassay (MBI) and its comparison to ELISA. The MBI technology involves the use of a Luminex 100 analyzer, associated software and fluorescently encoded microspheres. Each microsphere is labeled with a distinguishable fluorophore that allows it to be assigned or gated to a

particular region of the scanner. Antibodies, specific for the protein of interest, are covalently linked to beads of a unique fluorescent region. The combination of different beads allows the user to simultaneously measure various protein markers of interest. Assays were developed for Th1/Th2 markers (IL-2, IL-4, IL-5, IL-10, IFN- γ) as well as proinflammatory/sepsis markers (IL-1 β , IL-6, GM-CSF, MIP-2, TNF- α). Comparisons to ELISA show comparable specificity, sensitivity (< 10 pg/mL), and recovery in tissue culture or serum matrices (> 80 percent). Correlation to ELISA on natural samples generally exceeded 0.80 indicating that this technology can be used with confidence that the quality of information gathered will correspond well to observations made using traditional ELISA.

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Macrophage inhibitory cytokine-1 (MIC-1) in epithelial neoplasia

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Macrophage inhibitory cytokine-1 (MIC-1) is a recently described divergent member of the TGF- β superfamily, first cloned on the basis of increased mRNA expression associated with human macrophage activation. As *in situ* hybridization of mouse tissues has demonstrated widespread epithelial expression of MIC-1, we have hypothesized that MIC-1 may play a role in epithelial malignancies. In order to investigate this possibility, we assessed the presence of MIC-1 in epithelial tumor biopsies, the secretion of MIC-1 by tumor cell lines, and evaluated the MIC-1 serum levels in a small number of patients with metastatic carcinoma. The presence of MIC-1 was clearly demonstrated immunohistochemically in tissue samples from three common epithelial malignancies: colorectal, breast and prostate. It was largely undetectable in the corresponding normal tissues. MIC-1 was also secreted in large amounts by several tumor cell lines and its presence could be detected in greatly increased quantities in the serum of patients with metastatic epithelial malignancies. In the small number of patients studied, elevation of MIC-1 was positively correlated with plasma CEA levels in colorectal carcinoma, suggesting a relationship to disease stage. MIC-1 is a potential novel tumor marker that is easily quantified in serum and this study provides strong support for undertaking more comprehensive investigations of both the clinical utility of MIC-1 and its role in the development of cancer.

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A Theoretical Model for the Simulation of Sepsis

Rukmani Kumar, Carson C. Chow, Gilles Clermont, Yoram Vodovotz. University of Pittsburgh.

Sepsis in critically ill patients involves a well-orchestrated innate immune response. Imbalances in this response are thought to contribute to organ failure and death. Good rationales guiding the choice and timing of therapies that modulate this response are lacking. Using known interactions between key components of the innate immune system, we developed a two-compartment mathematical model of the early response to infection. The change of concentration over time of components such as bacteria, macrophages, neutrophils, pro- and anti-inflammatory cytokines (e.g. TNF- α and TGF- β 1, respectively), immune effector mechanisms (e.g. nitric oxide [NO]), tissue damage, and blood pressure were modeled with differential equations. We manipulated model parameters based on literature data and recreated several scenarios of both basic and clinical interest. TGF- β 1 suppresses NO production both *in vitro* and *in vivo*. TGF- β 1 transgenic mice exhibit 4-8 fold elevation in plasma active TGF- β 1 levels at baseline (Sanderson et al, PNAS 92:2572), and blunted systemic NO production (plasma NO $_2^-$ + NO $_3^-$) following intraperitoneal challenge with LPS (Vodovotz et al J. Leukoc. Biol.

63:31). We simulated this scenario by enhancing the baseline levels of our generic anti-inflammatory cytokine, resulting in a prediction of elevated plasma NO $_2^-$ + NO $_3^-$ following infection. Anti-inflammatory therapy is possibly useful in improving the outcome of patients with sepsis. We simulated this therapy by neutralizing the generic pro-inflammatory cytokine at various times and to various degrees. We noted a limited time span following infection where anti-inflammatory therapy is successful in avoiding mortality. Other scenarios, including antibiotic administration, antibiotic resistance, and chronic granulomatous disease were also simulated. Once calibrated to experimental data, such models might prove useful in developing therapeutic strategies and furnishing quantitative predictions in individual patients.

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WITHDRAWN

ONCOGENESIS (314-316)

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The cytokine profile in TPA-induced tumor promotion and its inhibition by perilla

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TPA (12-*O*-tetradecanoylphorbol-13-acetate), typically used as a tumor promoter, also causes an experimental acute dermatitis. But the cytokine profile which correlates with these phenomena is not well identified. We investigated here the inflammatory cytokine productions on skin spread with TPA. When TPA was spread on mouse ears, ear edemas with flare and itching were occurred. The ears were homogenized and centrifuged and the cytokine

contents of the supernatant were analyzed by ELISA. In normal ear homogenate, high level of IL-1 α and low level of IL-6, IFN- γ , and TNF- α were detected, but IL-1 β could not be detected. When TPA was spread on mouse ears, the local level of IL-1 α , IL-6, IFN- γ , and TNF- α was dose- and time-dependently augmented and IL-1 β was dramatically increased. The duration of local production of IL-1 α , IL-6, and IFN- γ were a few hours, and those of IL-1 β and TNF- α were at least more than 24 hours, respectively. These features markedly appeared with repeated applications of TPA which assumed the tumor promotion and the levels of each cytokines were further augmented. We also found that the oral administration of the extracts of *Perilla frutescens* leaves, which are used for daily food material in Japan, can inhibit tumor promotion. Oral administration of perilla leaf extract also inhibited TPA-induced ear edema and simultaneously inhibited the local production of IL-6, IFN- γ , and TNF- α . The essential aspect of tumor promotion is thought to be certainly the chronic inflammation which accompanies with the production of some inflammatory cytokines. We consider therefore that the inhibition of inflammatory cytokines by daily food such as perilla may provide the cancer chemoprevention.

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THE GLOBAL SUPPRESSION OF CYTOKINE GENES IN PROSTATE CANCER CELL LINES IS ASSOCIATED WITH ANDROGEN-SENSITIVITY

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We hypothesized that the process of malignancy in prostate epithelium leads to suppression of pro-inflammatory cytokine production thereby inhibiting local immune and inflammatory responses. Utilizing TaqMan real-time quantitative RT-PCR, primary cultures of benign prostate epithelial cells, PrEC, revealed constitutive expression of pro-inflammatory cytokine genes but not in LNCap cells, an androgen-sensitive (AS) cell line. In DU 145 or PC-3, androgen-insensitive (AI) malignant prostate cell lines, expression of cytokine genes was also reduced but not as global as in LNCaP. To investigate the correlation between cytokine gene expression and androgen-sensitivity we evaluated the gene expression profile in AS cell lines, MDA Pca-2b and 22Rv1. In contrast to AI cell lines, each of new AS cell lines exhibited global suppression of pro-inflammatory cytokine genes similar to previously observed in LNCaP. Expression of IL-1 α , IL-1 β , TNF α , IL-6, IL-8 and GM-CSF genes in MDAPca-2b and 22-Rv1 was below the sensitivity level. Expression of IL-18 was detectable but weak, whereas the gene expression of caspase-1 was not observed. These data reveal significant differences in cytokine gene expression profiles of AS and AI cell lines. The observed correlation between androgen-sensitivity and suppression of cytokine genes in prostate cancer cell lines may reflect one of potential immune escape mechanisms in prostate cancer.

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Deregulation of Notch2 signaling in B-CLL

Rainer Hubmann, Josef Schwarzmeier, Medhat Shehata, Martin Hilgarth, Markus Dürchler. LBI for Cytokine Research, University of Vienna Medical School. Waehringer Guertel 18-20, PO Box 16, A-1097 Vienna, Austria. The overexpression of the multifunctional cytokine CD23 is one of the major characteristics of B-CLL cells. Besides the prognostic potential of its soluble cleavage product, sCD23, selective expression of the CD23a isoform is concurrent to a state of B-CLL cell survival, thereby providing a link between CD23a and the malfunction of apoptosis characteristic for this neoplastic B-cell type. By electrophoretic mobility shift assays, we identified a transcription factor complex (C1) which binds sequence specific to one known and four newly identified putative CBF1 recognition sites in the CD23a core promoter region. The significance of this complex was underlined by the fact, that in B-cell samples the intensity of C1 correlated with their respective levels of CD23a transcription. Furthermore, using Epstein Barr virus (EBV) infected B-cells as a model for CBF1 mediated CD23a expression, C1 was found to be

EBV inducible. Supershift assays revealed that the nuclear form of Notch2 is a component of C1 in B-CLL cells, supporting a model in which Notch1C activates transcription by binding to CBF1 tethered to DNA. Finally, RT-PCR analysis indicated that the Notch2 oncogene is overexpressed in B-CLL cells. These data suggest that deregulation of Notch2 signaling, which is known to inhibit differentiation and apoptosis, plays a pivotal role in the upregulation of the CD23 gene in B-CLL cells.

DISEASE ROLES OF CYTOKINES (317-333)

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CLINICAL AND BIOLOGICAL ELEMENTS REGARDING THE NETWORK OF CYTOKINES IN MULTIPLE MYELOMA

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The study of the network of cytokines seems to be useful for the identification of growth factors in multiple myeloma.

Plasma cells may produce IL-6 by autocrine mechanism whereas paracrine mechanism is involved in the IL-6 production by bone marrow stromal cells. The involvement of IL-6 in the biology of multiple myeloma is based on its ability to induce the differentiation of myeloma plasmablastic cells into mature malignant plasma cells. Differential diagnosis between multiple myeloma and monoclonal gammopathies of undetermined significance (MGUS) is generally based on clinical and laboratory parameters. Nevertheless, the evaluation of the serum level of IL-6, C reactive protein, soluble IL-6 receptor, soluble IL-2 receptor together with the activity exerted by IL-3 and IL-4 on some cellular subsets may constitute an additional element in the differential diagnosis of border-line cases.

Serum levels of IL-6, soluble IL-6 receptor (sIL-6R), soluble interleukin-2 receptor (sIL-2R) and the expression of membrane-bound IL-2 receptors, both on bone marrow plasma cells and on peripheral blood mononuclear cells are correlated with the disease activity and the disease stage. In addition, IL-6 and sIL-6R serum level are correlated with the duration of disease-free survival as a high value at the time of diagnosis is connected to a short duration of survival.

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Soluble Macrophage Colony-Stimulating Factor Receptor (M-CSFR) in Children Hematopoietic Disorders

Xiaojin Sha, Qing Rao, Yuhua Song, Kefu Wu*. Institute of Hematology, Chinese Academy of Medical Science, Tianjin, P.R. China. Macrophage colony-stimulating factor (M-CSF) is a multi-functional cytokine appeared in serum and urine. It is an important growth factor for monocytic lineage and also important inflammatory factor for some diseases. The effects of M-CSF are mediated by its receptor (M-CSFR). In addition to the membrane receptor, soluble isoform in serum and urine has been detected by specific sandwich ELISA that was established in our lab recently. In this report the presence of M-CSFR in children sera was investigated. The results demonstrated a steady level of M-CSFR in 26 normal children sera (0.54 \pm 0.54 ng/ml). The serum M-CSFR level in 208 children with hematopoietic disorders was also studied. Serum M-CSFR levels in 92 acute lymphoid leukemia (ALL) patients (0.14 \pm 0.19 ng/ml, p=0.001) and 31 acute non-lymphoid leukemia (ANLL) patients (0.19 \pm 0.19 ng/ml, p=0.009) were reduced compared with normal group. No significant difference was found between the ALL and ANLL patients. For ALL patients, serum M-CSFR level of standard risk group was higher than the higher risk group (0.20 \pm 0.22 ng/ml vs 0.06 \pm 0.16 ng/ml, p=0.012). No significant differences were found between male and female, T-ALL and B-ALL. No correlations were discerned between the serum M-CSFR level and peripheral white blood cell count, the total count of granulocyte and monocyte in patients with ALL and ANLL. Serum M-CSFR level in 43 patients with aplastic anemia (AA) (0.24 \pm 0.33 ng/ml, p=0.015) were lower. In contrast, higher levels of serum M-CSFR were shown in 42 patients with Idiopathic thrombocytopenic purpura (ITP) (1.06 \pm 1.17 ng/ml, p=0.016) and the severe groups of ITP (platelet < 25 \times 10⁹/l).

9/L, 1.49 ± 1.33 ng/ml, $p=0.003$). No significant difference was found between the mild group (platelet $> 25 \times 10^9$ /L, 0.54 ± 0.68 ng/ml) and the control. Finally, the molecular weight of M-CSF α in serum is about 90 kD which was identified by immunoprecipitation and Western blot. Our data suggests that M-CSF α might play some roles in some children hematopoietic disorders, especially in ITP and AA patients.

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Adenoviral expression of murine Oncostatin M induces inflammation and bone apposition in joints of IL-1, IL-6 and TNF- α deficient mice.

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The IL-6 family member Oncostatin M (OSM) is expressed in the joints of rheumatoid arthritis patients. Murine OSM was expressed in the joints of naive mice by the adenoviral vector AdmuOSM. This induced an inflammation that lasted for weeks, confirming previous data. Expression of IL-1, IL-6 and TNF- α was greatly enhanced in the synovium as shown by RT-PCR. To determine the role of these cytokines in the AdmuOSM induced inflammation, we injected this vector in mice deficient for IL-1, IL-6 or TNF- α . Although the acute inflammation was lower in IL-1 and TNF- α deficient mice, inflammation at day 14 was not reduced compared to wildtype mice. Also did these mice not differ from wildtype mice in cartilage proteoglycan depletion. This depletion was not only observed in articular but also in epiphyseal cartilage of all mice examined. In the epiphysis also the integrity of the matrix was disrupted. Early during the inflammation, the periosteum became activated and new bone apposition took place in an irregular way. The latter was not caused by erosion of the new bone as no TRAP-positive cells were observed at the site of bone apposition. These results suggest an important and independent role for OSM in joint pathology and show that overexpression leads to damage to the epiphysis and bone apposition.

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Dual Roles of IL-6 in Airway Hypersensitivity Suggested by Enhanced Mucus Secretion and Reduced Inflammatory Cell Infiltration in Ovalbumin-challenged IL-6-deficient Mice

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To clarify the role of IL-6 in the allergic airway inflammation, we challenged wild-type and IL-6-deficient mice with ovalbumin (OVA) inhalation after the sensitization. OVA challenge induced a considerable intrapulmonary eosinophil and lymphocyte infiltration and airway hyperresponsiveness in wild-type mice with increased serum IgE levels and enhanced intrapulmonary mRNA expression of Th2 cytokines, IL-5 and IL-13. IL-6-deficient mice developed a similar level of airway hyperresponsiveness despite an attenuated eosinophil and lymphocyte infiltration and lower serum IgE levels after OVA challenge. Treatment of wild-type mice with a neutralizing anti-IL-6 monoclonal antibody during the sensitization mimicked the phenotypes of OVA-challenged IL-6-deficient mice in terms of lower serum IgE levels and attenuated eosinophil infiltration. After OVA challenge, intrapulmonary IL-5 and IL-13 mRNA expression was less evident in IL-6-deficient mice than wild-type mice, while intrapulmonary IFN- γ mRNA expression was more enhanced in IL-6-deficient mice. However, after OVA challenge, IL-6-deficient mice exhibited more exaggerated goblet cell metaplasia than wild-type mice. Thus, endogenous IL-6 may have conflicting roles in OVA-induced airway hypersensitivity consisting of induction of Th2 polarization and eosinophil infiltration, and inhibition of goblet cell metaplasia.

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REGULATION OF MACROPHAGE CHEMOKINE EXPRESSION BY HYPOXIA: A POTENTIAL ROLE IN THE ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS)

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We have previously reported elevated bronchoalveolar lavage (BAL) IL-8 levels in patients at-risk of ARDS (Lancet 1993; 341: 643). We now report an extended study of trauma patients and confirm raised BAL IL-8 levels are associated with ARDS progression ($n=56$, $P < 0.01$). This association was not seen with a range of other chemokines, cytokines or with endotoxin levels. Raised IL-8 levels were detected 95 mins following trauma and the macrophage identified as a potent source. Patient hypoxia (reduced $\text{PaO}_2/\text{FiO}_2$) on presentation correlated with raised BAL IL-8 ($r=-0.56$, $P < 0.01$). We postulated that acute hypoxia upregulates IL-8 synthesis in macrophages. Macrophages cultured from peripheral blood mononuclear cells from healthy volunteers were exposed to hypoxia or normoxia for up to 2 hrs. IL-8 protein and mRNA expression were significantly increased under hypoxic conditions (1.8 and 2.5 fold respectively). In contrast, hypoxia inhibited IP-10 (100), RANTES (62 ± 12), MIP-1 α (52 ± 10), MIP-1 β (76 ± 9), MCP-1 (100) and TNF- α (38 ± 8) mRNA expression (mean % inhibition of normoxia \pm SEM, $P < 0.02$). Hypoxia rapidly (< 15 mins) activated AP-1, C/EBP but not NF- κ B expression. Hypoxia activated HIF-1 α , but cobalt and desferrioxamine (HIF-1 α -inducing hypoxia mimics) did not induce IL-8, implying a HIF-1 independent mechanism in IL-8 regulation. Both the pattern of chemokine/cytokine expression and transcription factor activation following hypoxia was different to that seen with LPS. **Conclusion.** Acute hypoxia upregulates IL-8 in macrophages in association with a novel pattern of transcription factor activation. The selectivity and pattern of activation differs from that seen with endotoxin, suggesting alternative pathways to the development of lung injury. undefined

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Neutralisation of endogenous IL-18 activity is a disease-modifying therapy in the collagen-induced model of arthritis

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Two distinct IL-18 neutralising strategies, i.e. a rabbit polyclonal anti-mouse IL-18 IgG and a recombinant human IL-18 binding protein (rhIL-18BP), were used to treat collagen-induced-arthritis (CIA) mice after clinical onset of disease. The therapeutic efficacy of neutralizing endogenous IL-18 was assessed using different pathological parameters of disease progression. The clinical severity in mice undergoing collagen-induced arthritis was significantly reduced after treatment with both IL-18 neutralising agents compared to placebo treated mice. Attenuation of the disease was associated with reduced cartilage erosion evident on histology. The decreased cartilage degradation was further documented by a significant reduction in the levels of circulating cartilage oligomeric matrix protein (an indicator of cartilage turnover). Both strategies efficiently slowed disease progression, however, only anti-IL-18 IgG treatment significantly decreased an established synovitis. Serum levels of IL-6 were significantly reduced with both neutralizing strategies. In vitro, neutralising IL-18 resulted in a significant inhibition of TNF- α , IL-6 and IFN- γ secretion by macrophages. These results demonstrate that neutralising endogenous IL-18 is therapeutically efficacious in the murine model of collagen-induced-arthritis. IL-18 neutralising antibody or rhIL-18BP could therefore represent new disease-modifying anti-rheumatic drugs that warrant testing in clinical trials in patients with rheumatoid arthritis.

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Interleukin (IL)-18/IL-18 Binding Protein Signalling Modulates Atherosclerotic Lesion Development and Stability

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Atherosclerosis is the leading cause of mortality in industrialised countries and carries an important socio-economic burden. Unabated inflammatory mechanisms are responsible for changes in atherosclerotic plaque composition leading to plaque disruption and to the occurrence of acute ischemic syndromes, namely, myocardial infarction and stroke 1. Interleukin (IL)-18 is an inducer of IFN- γ 2 with potent activities on inflammatory and vascular cells and is thought to contribute to the pathogenesis of chronic immuno-inflammatory processes 3,4. We have recently detected increased production of IL-18 by macrophages and smooth muscle cells in unstable human atherosclerotic plaques that were responsible for strokes compared with stable plaques from asymptomatic patients 5. We now extend these findings and show increased levels of IL-18 in the circulating blood of patients with acute ischemic coronary syndromes (unstable angina and myocardial infarction) compared with non-ischemic patients. Taken together, these results obtained in humans suggest a potential role for IL-18 in plaque destabilisation. An endogenous IL-18 binding protein (IL-18BP) that neutralises IL-18 has been identified 6. However, the role of IL-18BP in the modulation of atherogenesis and other chronic immuno-inflammatory diseases *in vivo* is currently unknown. In this study, we show that *in vivo* electrotransfer of an expression plasmid DNA encoding for murine IL-18BP prevents fatty streak development in the thoracic aorta of apoE knockout mice and slows progression of advanced atherosclerotic plaques in the aortic sinus. More importantly, transfection with the IL-18BP plasmid induces profound changes in plaque composition (decrease in macrophage, T cell, cell death and lipid content and increase in smooth muscle cell and collagen content) leading to a stable plaque phenotype. These results identify for the first time a critical role for IL-18/IL-18BP regulation in atherosclerosis and suggest a potential role for IL-18 inhibitors in reduction of plaque development/progression and promotion of plaque stability.

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Production of cytokines by erythroid nuclear cells of bone marrow isolated from patients with myelodysplastic syndromes.

T.V. Injelevskaya, S.V. Sennikov, V.A. Kozlov, I.B. Kovinev, M.I. Loseva. Institute of Clinical Immunology SB RAMS, The Department of Hospital therapy of propanthology of Novosibirsk Medical Academy. Previously the presence of IL-1, IL-2, IL-6, IL-4, IL-10, TNF- α , IFN- γ and TGF- β 1 was found in conditional media of erythroid nuclear cells (ENC) of bone marrow (BM) from healthy donors. The level of cytokine production has changed after addition erythropoietin to the cells. Patients with myelodysplastic syndromes (MDS) have diserythropoiesis in BM. Cytokines and growth factors imbalance take place in MDS pathogenesis as well as an increase of cell apoptosis. We have assumed that cytokine-synthesizing activity of BM ENC will change at MDS. Erythroid cells were isolated by positive and negative selection. ENC of human BM (10^6 cell/ml) were cultivated 24 hours. Conditional media of erythroid cells was isolated and concentration of cytokines measured by electrochemiluminescence method by using poly- and monoclonal antibodies. We have found that BM ENC produce IL-1 β , IL-2, IL-6, IL-4, IL-10, TNF- α , IFN- γ and TGF- β 1. Significant changes in cytokine production were found for TNF- α , IL-10, IL-4. The level of TNF- α was higher and level of IL-4, IL-10 lower in conditional media of BM ENC from patients with MDS than in conditional media of BM ENC from of the healthy donors. Increase of TNF- α who provoke apoptosis may play a role in MDS pathogenesis.

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ADENOVIRAL EXPRESSION OF IL-10 PROMOTES A DOSE-DEPENDENT SURVIVAL ADVANTAGE TO ZYMOSAN-INDUCED ARDS

M.E. Murday, F.R. Bahjat, R.M. Minter, R. Ungaro, J. DeBernardis, L.L. Moldawer. Department of Surgery, University of Florida College of Medicine. Use of gene therapy to target IL-10 delivery to the lung has been promulgated as a means to treat ARDS. We recently demonstrated that intratracheal (it.) administration of low doses of adenoviral vectors expressing human (Ad/hIL-10) or viral IL-10 (Ad/vIL-10) conferred a survival advantage in mice treated with ARDS produced by intraperitoneal zymosan. The purpose of this study was to determine the optimal dose of Ad/vIL-10 or Ad/hIL-10 producing the maximal survival advantage in zymosan treated mice. **Methods:** C57/BL6 female mice were pretreated it. 10^7 , 10^8 or 10^9 particles of Ad/hIL-10, Ad/vIL-10, Ad/empty or buffer alone. Twenty-four hours after it. Injection, the animals were intraperitoneally injected with zymosan. Weight and survival curves were generated. Bronchoalveolar lavage, serum and lung tissue were collected from animals on days 1, 5, 10 and 18 and examined for inflammatory cytokines and IL-10 expression. **Results:** As expected, levels of recombinant IL-10 in the lung and BAL correlated with the dose administered. Serum levels of vIL-10 and hIL-10 were not measurable at the 10^7 dose; the dose that conferred survival advantage. Human IL-10 levels were detected in the serum at both the 10^8 and 10^9 dose; these doses were not associated with improved mortality. Conversely, serum vIL-10 levels were detectable only 10^9 dose. The difference in serum detection of recombinant viral and human IL-10 is consistent with the demonstration of greater tissue compartmentalization with vIL-10. A survival advantage was greatest for animals pretreated with Ad/vIL-10 at the 10^7 dose; however, 10^8 dose of Ad/vIL-10 and 10^9 dose of Ad/hIL-10 conferred some survival advantage. Adenovirus expressing IL-10 delivered at doses of 10^9 resulted in increased mortality, possibly due to systemic levels of IL-10. **Conclusions:** We conclude that adenoviral delivery of IL-10 can improve outcome in zymosan-treated mice with ARDS, but the responses are dose-dependent.

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Macrophage inflammatory protein 1 α (MIP-1 α) inhibits the production of IL-4 by splenic T cells stimulated with monocyte chemoattractant protein 1 (MCP-1)

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A predominance of Th2 responses is commonly observed in animals and patients with severe thermal injuries. Th2 cells have been identified as the cells responsible for the increased susceptibility of thermally injured mice to infections with herpes simplex virus type 1 (HSV-1) and *Candida albicans*. MCP-1 has been described as an inducer of the Th2 cell generation, and MIP-1 α has been shown to be a stimulator of the Th1 responses. Therefore, in the present study, the effect of MIP-1 α on the MCP-1 associated-polarization of Th2 responses was examined. T cells were prepared from spleens of BALB/c mice by T cell enrichment columns. These cells (2×10^6 cells/ml) were stimulated with MCP-1 (100 ng/ml) in combination with anti-CD3 mAb (2.5 μ g/ml) for 3 hrs. After the stimulation, cells were washed and recultured with the fresh medium for an additional 48 hrs in the presence of 0.1 to 100 ng/ml of MIP-1 α . As a representative Th2 cytokine, IL-4 was measured in the culture fluids of these cells. Also, IFN- γ in these cultures was assayed as a representative cytokine for the Th1 responses. IL-4 and IFN- γ were measured by ELISA. Splenic T cells stimulated with MCP-1 and anti-CD3 mAb produced 52 pg/ml of IL-4 into their culture fluids. However, after the re-cultivation with 10 ng/ml of MIP-1 α , IL-4 was not produced by these T cells. The IFN- γ production was stimulated by IL-12 (10 U/ml) in cultures of T cells that were stimulated with MCP-1 in combination with anti-CD3 mAb and recultured with MIP-1 α , while T cells treated with MCP-1 and anti-CD3 mAb did not produce IFN- γ when they were stimulated with IL-12. These results suggest that MIP-1 α may have a capability to regulate the Th2 cell generation stimulated by MCP-1. MIP-1 α may improve the resistance of thermally injured patients to HSV-1 or *C. albicans* infection.

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Glycyrrhizin inhibits the production of monocyte chemoattractant protein 1 (MCP-1) in cultures of T cells and macrophages.

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MCP-1 plays an important role on the recruitment and activation of monocytes during inflammatory responses. Recently, MCP-1 has been described as a stimulator of the Th2 cell generation. Since the polarization of Th2 responses has been inhibited by glycyrrhizin (GR), in the present study the inhibitory effect of GR on the MCP-1 production was investigated in cultures of murine splenic macrophages and T cells. GR, an active component of licorice roots, was supplied by Minophagen Pharmaceutical Co. Ltd., Tokyo, Japan. To induce the production of MCP-1, 1×10^6 cells/ml of macrophages and T cells, isolated from spleens of BALB/c mice (8-week-old), were stimulated with 10 ng/ml of IL-1, TNF- α , IL-4, IL-10 or IL-13 in the presence or absence of 0.1 to 100 μ g/ml of GR for 6 to 48 hrs. In some experiments, cells previously treated with the stimulators were cultured with GR for 12 hrs. Culture fluids harvested were assayed for MCP-1 by ELISA. Without any stimulation, macrophages and T cells produced less than 0.4 ng/ml of MCP-1 into their culture fluids. However, 6 to 11 ng/ml of MCP-1 were produced by macrophages stimulated with IL-1, TNF- α , IL-4, IL-10 or IL-13 into their culture fluids. T cells stimulated with TNF- α , IL-4 or IL-10 produced 2 to 4 ng/ml of MCP-1 into their culture fluids. GR at 10 μ g/ml inhibited 85 to 92% of the MCP-1 production by these cells stimulated with various cytokines. The complete inhibition of the MCP-1 production was demonstrated when cytokine-stimulated macrophages or T cells were cultured with GR at a dose of 1 μ g/ml. In addition, 63 to 72% of the MCP-1 production was inhibited when macrophages and T cells previously stimulated with various cytokines were cultured with 1 to 10 μ g/ml of GR. These results indicate that GR has a capability to inhibit the MCP-1 production in cultures of murine macrophages and T cells stimulated with various cytokines. This finding may be involved in the inhibitory activity of GR on the Th2 polarization.

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Effect of macrophage inflammatory protein 1 α (MIP-1 α) on the CLP-induced infectious complications in a SCID-human chimera model of thermal injury.

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The majority of deaths in thermally injured patients are associated with infection rather than physical damage to the skin or an abnormal metabolism induced by the injury. Thermal injury induces the change of immune responses that influence the resistance of individuals against infectious complications. Recently, the impaired production of MIP-1 α was shown in patients with thermal injuries. Since MIP-1 α has a capability to stimulate host's anti-septic protective immunity, in the present study the effect of MIP-1 α on infectious complications in a SCID-human model of thermal injury was investigated. SCID mice were inoculated with a mixture of 5×10^6 cells/mouse of healthy donor's peripheral blood T cells (PBTC) and Th2 cells generated from the same PBTC, and they were designated as Th2/human SCID chimeras. The generation of Th2 cells was performed from PBTC after the stimulation with a mixture of PHA (2 μ g/ml), MCP-1 (20 ng/ml) and anti-IL-12 mAb (2 μ g/ml), as described previously. Immediately after the cell inoculation, Th2/human SCID chimeras were exposed to well-controlled cecal ligation and puncture (CLP). Then, these chimeras with CLP were treated with recombinant human MIP-1 α at doses of 1 to 200 ng/mouse immediately, 12 and 24 hrs after CLP. In the results obtained, Th2/human SCID chimeras were shown to be very susceptible to CLP than SCID mice inoculated with healthy donor's PBTC (control SCID chimeras). Whereas all of Th2/human SCID chimeras died within 36 hrs after CLP, 50% of control SCID chimeras survived. The impaired resistance of Th2/human SCID chimeras to CLP was recovered after the administration of MIP-1 α . Thus, after the administration of MIP-1 α at a dose of 100 ng/mouse, 80% of Th2/human SCID chimeras exposed to CLP survived, while 50% of healthy PBMC-SCID chimeras and 0% of Th2/human

SCID chimeras were survived after the same CLP. The susceptibility of thermally injured patients to infectious complications may be increased through the burn-associated impairment for the production of MIP-1 α .

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MTX and MPA are immunosuppressive by a different mechanism.

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Methotrexate (MTX) and mycophenolic acid (MPA) are used as immunosuppressive drugs in rheumatoid arthritis (RA) and graft rejection respectively. Their mechanism of action is not known. We have studied the effects of MTX and MPA on cytokine production in whole blood (WB) and isolated cells. Both drugs inhibit the production of IL-4, IFN γ , TNF α , GM-CSF and IL-13 after T-cell stimulation (aCD3/aCD28). When blood-derived T cells are activated in the presence of MTX, the cells enter the cell cycle and progress to S phase and mitosis. At that point, the cells die by apoptosis. Resting T cells are not affected. The mechanism of MPA is different. Cycling T cells become apoptotic after addition of MPA. However when resting T cells are activated in the presence of MPA, some activation markers (CD69 and CD25) are upregulated, but the cells stay small and do not enter S phase. This inhibition by MPA is reversible, even after fourteen days. We also studied the effect of MTX on cytokine production in cultures of WB from RA patients receiving MTX for the first time. Two hours after MTX administration in vivo, cytokine production was decreased. This decrease was prevented by the addition of an MTX antagonist (folinic acid) to the cultures. Preliminary results suggest that the decrease in cytokine production and the effect of folinic acid were correlated with the decrease in number of tender joints in the patient after 12 weeks. These results indicate that the effect of MTX and MPA on cytokine production might be an important aspect of their mechanism of action.

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INCREASED SERUM LEVELS OF INTERLEUKIN-1 RECEPTOR ANTAGONIST IN HUMAN OBESITY: A LINK TO THE RESISTANCE TO LEPTIN?

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Human monocytic cells express functional leptin receptors and leptin is capable of inducing the expression and secretion of the IL-1Ra which antagonizes leptin action at the hypothalamic level in rodents, thereby inducing leptin resistance. We therefore examined if IL-1Ra levels are associated with human hyperleptinemic states. Serum IL-1Ra levels were measured in 20 morbidly obese non-diabetic subjects (BMI 45 \pm 6 kg/m²; serum leptin 52 \pm 20 ng/ml) as well as in 10 age- and sex-matched lean controls (BMI 22 \pm 2 kg/m², serum leptin 7 \pm 4 ng/ml). Serum IL-1Ra concentrations were elevated 6.5-fold in the obese patients and correlated in a linear manner with the leptin levels (r^2 0.34, p = 0.01). When 15 of the 20 obese subjects underwent bypass surgery, mean BMI and leptin levels decreased to 33 \pm 7 kg/m² and 18 \pm 12 ng/ml, respectively, 6 months after the operation. Change in leptin concentrations was associated with reduction in IL-1Ra levels (p < 0.02). In conclusion, IL-1Ra levels are strongly elevated in human obesity, and its concentrations decrease after weight loss from bypass surgery. However, lean body mass and insulin resistance are better predictors of serum IL-1Ra concentrations than leptin levels, suggesting that additional metabolic factors control the secretion of this cytokine antagonist.

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EVALUATION OF THERAPEUTIC EFFECTS OF RECOMBINANT IL-1 RECEPTOR ANTAGONIST (IL-1RA) AND SUPEROXIDE DISMUTASE (SOD) ON A MODEL OF EXPERIMENTAL ARTHRITIS IN MICE

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Development of rheumatoid arthritis (RA) is associated with overproduction of some pro-inflammatory cytokines (TNF α , IL-1, IL-6, IL-15) and with a variety of other pathogenic factors. Reactive oxygen species also contribute to pathogenesis of RA being involved in damaging of own tissues. In this work the efficacy of human recombinant proteins IL-1ra and SOD, which block two potential RA targets, IL-1 and ROS respectively, were studied on a model of experimental arthritis in mice. Arthritis was developed in two different groups of C57Bl/6 mice by injecting either complete Freund's adjuvant or collagen II. The recombinant IL-1ra and SOD were respectively expressed in *E. coli*, *S. cerevisiae*, produced and purified to homogeneous consistence. IL-1ra and SOD at various dose intervals: 0.6-120 μ g and 1-10 μ g per mouse respectively, and both substances simultaneously at two fixed combined doses (0.6 and 6 of IL-1ra and 0.1 μ g of SOD) were injected into mice divided on several representative groups. The substances were injected daily during 3 weeks. Control groups involved intact animals and mice, which did not get any drugs. The strong anti-inflammatory activity of applied substances on decreasing of edema sizes, on diminishing of a pain syndrome, and on preventing of the pannus formation was observed. The therapeutic efficacy was manifested in normalization of blood indices and in prevention of cartilage and bone injuries based on immune state assaying and histological study in comparison with control animals with developed arthritis. When IL-1ra was applied alone doses 0.6 and 6 μ g per mouse were more effective than higher ones. The combined therapy against induced arthritis with IL-1ra and SOD did not demonstrate obvious synergistic effects.

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IL-15 plays an important role in the pathogenesis of HAM/TSP through activation of the T cells and persistence of antigen specific CD8 cells
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One of the hallmarks of the HTLV-I associated neurological disease, HAM/TSP, is the activation of T cells circulating in the peripheral blood. This is demonstrated by the spontaneous proliferation of the T cells in an ex vivo culture obtained from HAM/TSP patients. We have shown that this phenomenon is due to two independent autocrine/paracrine loops of IL2/IL2R and IL15/IL15R. We demonstrated that both IL-15 and IL15R expressions are up regulated by the HTLV-I encoded Tax protein. Another hallmark of this disease is the presence of an extraordinary high number of antigen-specific CD8 cells in the peripheral blood of these patients. The antigen specific CD8 cells recognize HTLV-I Tax peptide 11-19. These cells have been associated with the disease progression. We have shown that addition of antibodies which block the action of IL-15 in an ex vivo culture reduced the number of Tax-specific CD8 cells as determined by tetramer staining. These data suggest that IL-15 may play an important role in the pathogenesis of HAM/TSP disorder through activation of T cells and persistence of the Tax-specific CD8 cells.

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IL-1 α , but not IL-1 β , is required for contact-allergen-specific T cell activation during the sensitization phase in contact hypersensitivity
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Contact hypersensitivity response is a T cell-mediated cellular immune

response caused by epicutaneous exposure against contact allergens. In this reaction, after the first epicutaneous allergen sensitization, Langerhans cells catch allergens and migrate from the skin to draining lymph nodes and activate naive T cells. Although IL-1 is suggested to be involved in these reactions, the mechanisms have not been elucidated yet. In this report, to elucidate roles of IL-1 α and IL-1 β in contact hypersensitivity responses, we analyzed ear swelling in 2, 4, 6-trinitrochlorobenzene (TNCB)-induced contact hypersensitivity responses using gene-targeted mice. We found that ear swelling was suppressed in IL-1 α -deficient (IL-1 $\alpha^{-/-}$) mice but not in IL-1 $\beta^{-/-}$ mice. Langerhans cell migration from the skin into lymph nodes was delayed in both IL-1 $\alpha^{-/-}$ and IL-1 $\beta^{-/-}$ mice, suggesting that this defect might not be the direct cause for the reduced contact hypersensitivity responses in these mice. However, we found that the proliferative response of trinitrophenyl (TNP)-specific T cells after sensitization with TNCB was specifically reduced in IL-1 $\alpha^{-/-}$ mice. Furthermore, adoptive transfer of TNP-conjugated IL-1-deficient epidermal cells into wild-type mice indicated that only IL-1 α , but not IL-1 β , produced by antigen-presenting cells, Langerhans cells, in epidermal cells could prime allergen-specific T cells. These observations indicate that IL-1 α , but not IL-1 β , plays a crucial role in TNCB-induced contact hypersensitivity responses by sensitizing TNP-specific T cells.

CLINICAL IMPACT OF CYTOKINES (334-337)

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TACI-Ig Neutralizes Molecules Critical for B Cell Development and Autoimmune Disease: BLyS is required for B cell Development

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BLyS and APRIL have similar but distinct biological roles, mediated through two known TNF receptor family members, TACI and BCMA. We show that mice treated with TACI-Ig or TACI-Ig transgenic mice have fewer mature follicular and marginal zone B-2 cells. This block in development occurs specifically at the immature transitional T1 stage suggesting that factors neutralized by TACI-Ig play a role in the differentiation and/or survival of T1 B cells. In addition, these mice have fewer peritoneal B-1 B cells and lower levels of circulating immunoglobulin. In BLyS deficient mice, B cell development is blocked at the transitional T1 stage such that virtually no mature B cells are present. Peritoneal B-1 numbers are relatively unaffected by the loss of BLyS suggesting that additional molecules are essential for the development of this B cell population. The molecules neutralized by TACI-Ig are important for the development of an antigen-specific immune response. We demonstrate that TACI-Ig is capable of suppressing the development of pathogenic antibodies to collagen and the progression of disease in an animal model of rheumatoid arthritis. Taken together, these data suggest that BLyS is produced constitutively and is required for the development of pre-immune B cell populations. In addition, TACI-Ig neutralizes molecules essential for the development of pathogenic antibodies associated with disease. The mechanism of action for TACI-Ig in these processes will be discussed.

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Critical role for tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in innate immune surveillance against tumors

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TNF/TNFR family molecules are well known to play important roles in cell activation, survival, and death. We have reported that TRAIL (TNF-related apoptosis-inducing ligand) is constitutively expressed on murine NK cells in the liver and plays a substantial role in suppressing tumor metastasis. Freshly isolated NK cells, but not NKT cells or T cells, from the liver expressed cell-surface TRAIL, which was responsible for spontaneous cytotoxicity against TRAIL-sensitive tumor cells in vitro along with perforin and Fas ligand (FasL). Additionally, NK cells have been also implicated in innate immune surveillance against primary tumor development. Here we show that TRAIL plays a critical role in the NK cell-mediated and IFN- γ -dependent tumor

surveillance. Administration of neutralizing monoclonal antibody against TRAIL promoted tumor development in mice subcutaneously inoculated with TRAIL-sensitive tumor cell lines or a chemical carcinogen methylcholanthrene (MCA). Such a protective effect of TRAIL was at least partly mediated by NK cells and totally dependent on IFN- γ . In the absence of TRAIL, NK cells, or IFN- γ , TRAIL-sensitive sarcomas preferentially emerged in MCA-inoculated mice. These results indicated a substantial role of TRAIL as the effector molecule that eliminates developing tumors.

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MODULATION OF CHEMOKINE SIGNALING INVOLVES MULTIPLE RECEPTOR ASSOCIATED PROTEINS

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When the CXCR2 chemokine binds to its receptor, CXCR2, a number of downstream signals are generated that are required for mediation of chemokine biological activity. Some of these signaling events are involved in the desensitization, down regulation, and recycling of the receptor, while other signals are generated to evoke cell motility responses, gene expression, or escape from apoptosis. We have determined that ligand activation of CXCR2 results in its phosphorylation and this event is tied to desensitization of the receptor. After ligand activation, through interaction of the carboxyl-terminal domain of the receptor with AP-2 and β -arrestin, the receptor moves into clathrin coated pits, and then to early endosomes. The internalized receptor binds the phosphatase, PP2A, and becomes dephosphorylated in the endosomal compartment. When the process of receptor internalization is interrupted by mutation of the AP-2 and/or β -arrestin binding domains of the receptor, or by expression of dominant negative dynamin, the chemotactic response is markedly diminished. To determine why internalization might be required for chemotaxis, we have examined the effects of non-internalizing receptor on the localization and activation of Rho GTPase, PAK, PI3-kinase, and AKT. We hypothesize that receptor internalization is important in the establishment of the rhythm of intracellular localization of activated AKT or PAK during the chemotactic response. Since the availability of receptor to ligand and strength of ligand signaling is determined by the receptor internalization rate in combination with the recycling time of the receptor, deciphering these mechanisms for differences in regulation of trafficking chemokine receptors should provide key information for successfully developing chemokine receptor antagonists and for understanding more fully the processes involved in response to a chemokine gradient.

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Cancer Immunoediting by IFN γ and Lymphocytes

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Using neutralizing IFN γ -specific mAb or IFN γ insensitive mice that lack the IFN γ receptor or Stat1 we have shown that IFN γ functions to suppress the development of primary or transplanted carcinogen-induced or spontaneous tumors in immunocompetent mice. The tumor cell itself was found to be a key target of IFN γ 's actions and our data suggested that IFN γ functioned, at least in part, to enhance tumor cell immunogenicity. This work suggested a connection between IFN γ /Stat1-dependent tumor suppression and the controversial concept of cancer immunosurveillance. We have now evaluated the role of lymphocytes in suppressing tumor development using mice with a targeted disruption of the recombination-activating gene-2 (RAG2). RAG2^{-/-} mice lack all T, NKT and B lymphocyte populations but produce NK cells. RAG2^{-/-} mice developed significantly more tumors when challenged with the chemical carcinogen methylcholanthrene and exhibited higher rates of spontaneous primary tumor development compared to wild type syngeneic mice. Mice lacking both RAG2 and Stat1 developed an even wider array of spontaneous tumors. Tumors that developed in the absence of lymphocytes were more immunogenic than those that were produced in immunocompetent mice and exhibited a distinctive gene expression pattern. These results show

that the combined effects of IFN γ /Stat1 signaling and the immune system not only serve to impede tumor growth but also to shape the antigenic profile of tumors that develop in the host, a process that we term cancer immunoediting.

SLB MARIE T. BONAZINGA AWARD LECTURE (338)

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Antigen-specific T-cell mediated gene therapy with latent TGF- β 1 in the treatment of experimental allergic encephalomyelitis (EAE)

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Immunosuppressive cytokines such as TGF- β and IL-10 down-regulate autoimmune diseases. To determine whether locally accumulated T cells engineered to produce latent TGF- β 1 are capable of controlling autoimmune reactions and allergic inflammation, myelin basic protein (MBP)-specific and ovalbumin (OVA)-specific BALB/c cloned Th1 cells were transduced with cDNA for murine TGF- β 1. The transduced cells secreted 2-3 ng/ml of latent TGF- β 1, but maintained an otherwise unaltered cytokine secretion profile. When SJL x BALB/c F1 mice, immunized with proteolipid protein (PLP) in complete Freund's adjuvant (CFA), were injected with 3×10^6 cells from MBP-activated untransduced cloned Th1 cells, the severity of EAE was slightly increased. In contrast, MBP-activated (but not resting) TGF- β 1 transduced T cells significantly delayed and ameliorated the development of EAE in such recipients. TGF- β 1 transduced OVA-specific BALB/c Th1 clones did not influence EAE, even when the cells were re-exposed to OVA in the recipients. Spinal cords from mice 12-50 days after receiving TGF- β 1/MBP cells contained detectable TGF- β 1 cDNA, indicating persistence of the transduced cells. EAE relapses induced within the first 2 wks after TGF- β 1/MBP T-cell transfer by injection of bacterial superantigen or endotoxin, were suppressed, but no protection was observed against EAE relapses induced 8 wks after TGF- β 1/MBP T-cell transfer. However, EAE relapses induced late after immunization with PLP in CFA could be prevented by simultaneous injection of TGF- β 1/MBP T cells. In similar experiments, IL-10 transduced MBP-specific Th1 cells have been much less capable of ameliorating EAE than TGF- β 1/MBP cells, partially because the IL-10/MBP T cells seem less able to persist in the spinal cord of recipients.

INNATE IMMUNITY (339-346)

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TLR4, but not TLR2 agonists, activates phosphorylation of STAT1 α : Role of LPS-induced IFN- β .

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Although TLR2 and TLR4 agonists activate a common signaling pathway that results in translocation of NF- κ B, previous studies from our labs suggest that additional signaling pathways must be involved to account for differential gene expression and cytokine secretion seen in macrophages stimulated by TLR2 vs. TLR4 agonists. For example, we reported that certain genes, e.g., IP-10, MCP-5, and iNOS, are strongly inducible by TLR4, but not by TLR2 agonists. We compared the TLR4 agonist, *Escherichia coli* K235 LPS, to a panel of TLR2 agonists, including *Porphyromonas gingivalis* LPS, synthetic Pam3Cys, and *Mycobacterium tuberculosis*-derived STF, and have observed that phosphorylation of mitogen activated protein kinases is similarly induced. In contrast, *E. coli* LPS induces strong, delayed (2 h) STAT1 α phosphorylation that was strikingly impaired for all TLR2 agonists. Previous studies have suggested that STAT1 α phosphorylation is mediated by autocrine signaling of macrophages by enterobacterial LPS-induced type I interferons (IFN). IFN- β , an immediate-early, *E. coli* LPS-inducible gene, was poorly induced by all three TLR2 agonists. Monoclonal antibodies to murine IFN- β , but not IFN- α , blocked *E. coli* LPS-induced STAT1 α phosphorylation. Thus, TLR4-dependent activation of a gene subset whose induction is STAT1 α -dependent,

e.g., IP-10, MCP-5, and iNOS, is mediated by IFN- β . It is tempting to speculate that the failure of TLR2 agonists to activate the STAT1 α pathway may underlie the chronicity of bacterial agents such as *Porphyromonas* and *Mycobacteria*. AI-18797 (SV) and AI-47233 (MF)

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Effects of Acute Exposure to Nitrogen Dioxide on Human Bronchial Epithelial Cells: Involvement of Nitric Oxide and IL-8

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In our present study, normal human bronchial epithelial (NHBE) cells were used as an *in vitro* model of lung injury, to study the cellular changes following brief exposure to high dose of NO₂ (45 ppm), an oxidant gas. Since activated neutrophils (PMNs) play a major role in lung injury, adhesion of fMLF-stimulated PMNs to NHBE cells and the resultant PMN-mediated cytotoxicity, were also assessed in parallel. The NO₂ exposure of NHBE cells were carried out in the presence and absence of various pro-inflammatory cytokines (TNF- α , IFN- γ , IL-1 β), and changes in the levels of nitric oxide (NO), IL-8 and TNF- α were quantitated. Changes in cell morphology and viability were also assessed. The results demonstrate: a) NHBE cells generate low levels of NO (100-200 pmoles/10⁶ cells, measured as total nitrite), which, upon NO₂ exposure, increases by 4-5 fold within 6 hrs. This increase was reduced by 30-40%, in the presence of NO-inhibitors like L-NAME or L-NMMA. b) NHBE cells generated low levels of IL-8, which was increased 1-2 fold following NO₂ exposure. c) The nitrite levels were increased 40-70% in the presence of IFN- γ , IL-1 β , TNF- α and IFN- γ + TNF- α , in both unexposed and NO₂-exposed cells. d) In co-culture studies, prior treatment of NHBE cells with pro-inflammatory cytokines caused a significant increase (70-100%) in adherence of fMLF-stimulated PMNs to NHBE cells, and also resulted in a corresponding increase in PMN-mediated cytotoxicity of these cells. e) The inflammatory cytokines induced morphological changes in both unexposed and NO₂-exposed NHBE cells. These results suggest that NHBE cells are more susceptible to NO₂-mediated injury, when exposed to various inflammatory cytokines. Furthermore, both NO and IL-8 appear to be involved in the observed NO₂-mediated changes in NHBE cells, which is consistent with an active role of infiltrated PMNs in lung injury.

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Biased T Cell Responses by Manipulating Antigen Presenting Cells

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The development of CD4⁺ cells into polarized Th1 or Th2 type helper cells was examined, with a particular focus on the role that antigen presenting cells play in this response. Macrophages are antigen presenting cells (APCs) which are capable of secreting both pro-inflammatory and anti-inflammatory cytokines. It has been known that the cytokine milieu in which CD4⁺ cells are activated can play a major role in directing T cell development. Our previous studies have shown that ligation of the Fc γ receptors on macrophages in conjunction with an inflammatory stimulus results in an abrogation of IL-12 synthesis and a dramatic induction of IL-10. In the present study, antigen was directed to macrophage Fc γ receptors to determine whether this route of entry would influence the development of antigen specific CD4⁺ cells. We used an *in vitro* system composed of macrophages derived from bone marrow and CD4⁺ cells derived from the spleen of TCR transgenic mice. When APCs were stimulated with LPS and fed ovalbumin, they gave rise to antigen specific Th1-like CD4⁺ cells that produced IFN- γ and little IL-4. Alternatively, when stimulated macrophages were fed IgG-opsonized ovalbumin, they gave rise to Th2-like CD4⁺ cells that produced little IFN- γ and high levels of IL-4. This latter effect was the direct result of macrophage derived IL-10 produced in response to Fc γ receptor ligation, because macrophages from IL-10^{-/-} mice failed to direct a Th2 response, and unrelated immune complexes (IgG-erythrocytes) also promoted a Th2-type response. To determine the biological significance of the *in vitro* observations, *in vivo* studies were performed in which mice were injected with either ovalbumin or IgG-opsonized ovalbumin. The mice injected with IgG-opsonized antigen produced higher levels of total immuno-

globulin, which were predominantly of the IgG1 isotype, indicating a Th2 response. These results indicate that APCs can exert a profound effect on T cell development, and that the adaptive immune response can influence the innate immune response. They further imply that IgG itself is a strong stimulus for a Th2 type response.

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UPREGULATION OF IL-10R1 EXPRESSION IS REQUIRED TO RENDER HUMAN NEUTROPHILS FULLY RESPONSIVE TO IL-10

Marco A. Cassatella*, Luca Crepaldi*, Sara Gasperini*, Jose' A. Lapinet*, Federica Calzetti*, Cristina Pinardi*, Ying Liu§, Sandra Zurawski§, Rene' de Waal Malefyt§, Kevin W. Moore§. * Department of Pathology, General Pathology Unit, University of Verona, 37134 Verona, Italy, § DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304-1104, USA. We have recently shown that IL-10 fails to trigger Stat3 and Stat1 tyrosine phosphorylation in freshly isolated human neutrophils. Here we report that IL-10 can nonetheless induce Stat3 tyrosine phosphorylation and the binding of Stat1 and Stat3 to the Gamma-Interferon Response Region or the high-affinity synthetic derivative of the c-sis-inducible element in neutrophils that have been cultured for at least 3 hours with LPS. Similarly, the ability of IL-10 to upregulate suppressor of cytokine signaling (SOCS)-3 mRNA was dramatically enhanced in cultured neutrophils and, as a result, translated into the SOCS-3 protein. Since neutrophils' acquisition of responsiveness to IL-10 required de novo protein synthesis, we assessed whether expression of IL-10R1 or IL-10R2 was modulated in cultured neutrophils. We detected constitutive IL-10R1 mRNA and protein expression in circulating neutrophils, at levels which were much lower than those observed in autologous monocytes or lymphocytes. In contrast, IL-10R2 expression was comparable in both cell types. However, IL-10R1 (but not IL-10R2) mRNA and protein expression was substantially increased in neutrophils stimulated by LPS. The ability of IL-10 to activate Stat3 tyrosine phosphorylation and SOCS-3 synthesis, and to regulate IL-1ra and MIP-1 β release in LPS-treated neutrophils correlated with this increased IL-10R1 expression, and was abolished by neutralizing anti-IL-10R1 and anti-IL-10R2 antibodies. Our results demonstrate that the capacity of neutrophils to respond to IL-10, as assessed by Stat3 tyrosine phosphorylation, SOCS-3 expression, and modulation of cytokine production, is very dependent on the level of expression of IL-10R1.

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HYPOXIA AND IFN γ INHIBIT VIRAL REPLICATION IN MACROPHAGES

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Macrophages (M ϕ) play a key role in the immune responses exerting a wide repertoire of activities that depend on their location and activation status. IFN γ stimulates M ϕ antiviral activity. We demonstrated that hypoxia is a potent environmental factor which modulates the activity of IFN γ -primed M ϕ by regulating gene expression. In this study, we investigated whether hypoxia could affect the antiviral activity of IFN γ -treated M ϕ . The murine M ϕ cell line RAW264.7 was engineered to express the MoMLV-based pSun1 recombinant retrovirus encoding the EGFP reporter gene under the LTR promoter control. Engineered cells expressed high levels of pSun1 transcript that were slightly reduced when cells were treated with IFN γ . Inhibition of viral transcription was strongly enhanced upon exposure to hypoxia, occurring within 6 hr of culture and reaching peak levels at 18 hr. The negative regulatory role of hypoxia on retroviral expression was selective and could not be accounted for by induction of a deactivated cellular state, because hypoxia+IFN γ were still able to induce gene expression. Downregulation of intracellular viral protein levels by hypoxia+IFN γ was demonstrated by flow cytometric analysis of EGFP fluorescence and was associated with reduction of virus production and decreased viral infection. Interestingly, the effects of hypoxia +IFN γ were lineage-dependent, being exerted on other M ϕ cell lines but not on fibroblasts. These data emphasize the critical role of environmental stimuli, such as hypoxia, in host protection against viral infection.

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In vivo-derived Dendritic Cells show a different regulatory dependence for retaining immature function than GM-CSF differentiated bone-marrow-derived Dendritic Cells.

(1, 2) Michael Chattergoon and (2) Luis J. Montaner. D.V.M., D.Phil. (1) University of Pennsylvania, School of Medicine, Philadelphia PA. (2) The Wistar Institute, Philadelphia PA.

Since their discovery Dendritic cells (DC) have been extensively studied for their ability to stimulate naïve T-cells and initiate immune responses. Bone Marrow derived DC (BMDC), generated when murine bone marrow precursors are driven to differentiate along the myeloid lineage into DC by GM-CSF, have been used extensively to model and predict the behavior of DC in vivo. We have undertaken a direct comparison of directly isolated Splenic DC (SpDC) and BMDC. Our data suggests that freshly isolated SpDC and BMDC are both CD11c+, and are initially similar by expression of MHC-II (> 70% positive; BMDC MFI 6.7, SpDC MFI 8.0) and CD86 as well as endocytic potential (BMDC: > 92% endocytic, MFI FITC-Dextran =14.9; SpDC: > 95% endocytic, MFI FITC-Dextran =24.9). However, SpDC and BMDC differ in their dependence on GM-CSF to maintain their functional phenotype indicating that SpDC are not regulated in the same manner as BMDC. BMDC can be maintained in their initial differentiation state in the presence of GM-CSF, while SpDC increase their MHC-II expression (MFI day 0 = 6.7, day 3 = 15, $p < 0.005$) and reduce their endocytic ability (MFI FITC-Dextran day 0 = 25, day 3 = 5, $p < 0.005$) following 3 days in culture regardless of the presence of GM-CSF. Further, our data suggests that other splenocytes regulate the functional state of the DC in the spleen. >

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Contrasting role of heat shock protein(HSP)27 on dendritic cell(DC) differentiation vs maturation

A. De, K. Laudansky, C. Miller-Graziano. University of Rochester Med. Ctr. Unlike large HSPs which induce maturation of IL-4+GM-CSF(4+G) treated monocyte(MØ)-derived immature DC, no role for the small HSPs such as HSP27 in DC differentiation has been shown. We recently showed that exogenous addition of recombinant human HSP27 to human MØ induced exaggerated levels of IL-10 but minimal TNF α . Since IL-10 inhibits MØ to DC differentiation, HSP27 might also prevent MØ to DC conversion. MØ were differentiated to DC by 6-7 days of culture with 4+G(each 100ng/ml). TNF addition was used as a positive maturation factor for the last 2-3 days of culture. Hsp27(1µg/ml) was added to the MØ 3-5 hrs before addition of 4+G or added to immature DC (generated by culturing MØ with 4+G for 4-5 days). MØ / DC were characterized phenotypically by surface expression of CD14, CD86, CD1a and CD83 and functionally by secretion of IL-12 and induction of allogeneic T cell proliferation. Preincubation of MØ with hsp27 significantly inhibited the differentiation of MØ to immature DC (characterized both phenotypically and functionally). Simultaneous addition of α -IL-10 mAb and hsp27 could prevent the inhibitory responses of hsp27 on MØ to DC differentiation, indicating that hsp27 induced IL-10 was a critical mediator during hsp27 induced inhibition of MØ to DC differentiation. In contrast to hsp27's inhibition of MØ to DC differentiation, it significantly induced maturation of immature DC when added for the last 2-3 days of 7 days culture as compared to TNF α . These data indicate another novel function of hsp27: induction of DC maturation through a different mechanism than TNF α production.

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TRANSCRIPTIONAL REGULATION OF THE IL-18 BINDING PROTEIN GENE

Menachem Rubinstein, Daniela Novick, Vladimir Hurgin. Dept. of Molecular Genetics, The Weizmann Institute of Science, Rehovot 76100, Israel. The T-helper type 1 (Th1) immune response is a vital arm of the host defense against many pathogens; however, it is also associated with several autoimmune diseases and therefore needs to be tightly regulated. The cytokine IL-18,

originally termed IFN-gamma-inducing factor, is an early inducer of the Th1 cytokine response. IL-18-binding protein (IL-18BP) is a recently-identified circulating protein that tightly binds and neutralizes IL-18, thereby regulating the Th1 response. A low level of IL-18BP is constitutively expressed in the spleen, but to a much lesser extent in other tissues. IFN-gamma potently induces IL-18BP mRNA in vitro. We find that induction occurs also at the protein level. Other cytokines, including IL-18, IFN-alpha, IFN-beta, TNF-alpha, IL-1 and IL-2 do not induce IL-18BP, however, in cultured hepatocytes, TNF-alpha acts in synergy with IFN-gamma to induce significantly higher levels of IL-18BP. Maximal induction by IFN-gamma was obtained at 24 h and the induction was sensitive to cycloheximide, suggesting involvement of an intermediate IFN-gamma-induced transcription factor. A 2 kb genomic DNA upstream of the first exon of the human IL-18BP gene contains a promoter and an enhancer element, as determined by luciferase reporter vectors. This genomic DNA from both human and mouse origin contains an IRF-1/2-response element. Indeed, its co-expression with an IRF-1 expression vector induced high levels of luciferase activity. These data provide the mechanism of IL-18BP induction by IFN-gamma and support the notion that such induction represents a negative feedback loop, curbing extended IL-18 activity to reduce the risk of autoimmune damage.

CYTOKINES IN PATHOGENESIS (347-354)

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A role for BAFF in the development of Sjögren's syndrome through recruitment of B cells with a marginal zone-like phenotype

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BAFF (BlyS, TALL-1, THANK, zTNF4) is a secreted member of the TNF superfamily, that targets B-lymphocytes in particular and plays a role in their proliferation and survival. Mice transgenic (Tg) for BAFF develop an autoimmune condition similar to that of systemic lupus erythematosus (SLE). Excess BAFF in these transgenic mice possibly affects a critical tolerance checkpoint as immature splenic B cells differentiate, resulting to the expansion of both the immature transitional type 2 (T2) and the marginal zone (MZ) B cell compartments in secondary lymphoid organs. We now demonstrate that BAFF Tg mice, as they age, develop a secondary pathology to their lupus-like condition, which is reminiscent of Sjögren's syndrome, an autoimmune disorder affecting salivary and lacrimal glands. Polyclonal B lymphocyte activation and production of various autoantibodies are particularly intense in Sjögren's syndrome. We characterized a new B cell population with a MZ-like phenotype infiltrating salivary glands of BAFF Tg mice, which may play a pathological role. Importantly, we have been able to demonstrate that Sjögren's syndrome in humans also correlates with elevated levels of serum BAFF as well as production of BAFF in inflamed salivary gland biopsies from these patients. This work suggests that specific alteration of B cell function induced by excess production of BAFF is a critical event contributing to the pathogenesis of Sjögren's syndrome in mice and humans. Moreover, this work outlined a new population of B cells recruited to the targeted glands which may also be an important factor in the pathogenesis of certain autoimmune diseases.

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STAT1 PROTECTS AGAINST THE NEUROTOXIC ACTIONS OF IFN- α MEDIATED BY DISTINCT SIGNALING MECHANISMS IN VIVO.

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Transgenic mice (termed GIFN) that produce IFN- α 1 from astrocytes are protected from infection with neurotropic viruses but develop progressive neurodegenerative disease in association with upregulated expression by neurons of a number of IFN-regulated genes and activation of the key IFN-signaling molecules STAT (ST)1, 2 and IRF-9. Here we examined the

regulation and the role of the JAK/STAT signaling pathway in mediating the actions of IFN in the CNS. Unexpectedly, G1FN mice deficient for ST1 but not ST2 had accelerated and more severe neuropathological alterations than STAT replete G1FN mice. CNS expression of the IFN-regulated genes including e.g. PKR and OAS as well as, ST2 and IRF9, was not increased in G1FN/STAT1 KO mice consistent with the absence of STAT1-dependent IFN-regulated gene transcription. By immunoblotting, no significant changes in either the phosphorylated or total protein was detected for SAPK/JNK, ERK, p38MAPK and I κ B- α in brain from G1FN/ST1KO mice compared with G1FN mice. Gene chip analysis identified 32 genes involved in acute-phase response, inflammation, signal transduction and calcium handling whose expression was increased by > 3-fold in G1FN/ST1 KO mice compared with G1FN mice. The expression of 40 genes, including many known to be IFN-induced ST1 dependent, was decreased > 3-fold in the G1FN/ST1 KO mice. Thus: (1) ST1 alone mediates protection against IFN- α neurotoxicity, (2) the neurotoxic actions of IFN- α are mediated by an as yet unidentified alternative signaling pathway, (3) stress kinase and NF- κ B signaling are not responsible for the accelerated neuropathogenesis in G1FN/ST1KO mice, and (4) gene expression profiling highlights significant alterations in IFN actions in the brain without ST1 offering insights as to possible candidate genes that may mediate IFN- α neurotoxicity. Support: NIH grants MH 62231 and MH 62261.

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The role of the G-protein coupled receptor FPRL1 in Alzheimer's disease: Its relevance to cellular up-take of β amyloid peptide and fibrillar formation by macrophages

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The 42 amino acid form of β amyloid (A β 42) plays a pivotal role the activation of mononuclear phagocytes and neurotoxic effects in Alzheimer's disease (AD). Our recent study revealed that FPRL1, a G-protein coupled receptor, mediates the chemotactic and activating effect of A β 42 mononuclear phagocytes (monocytes and microglia), suggesting that FPRL1 may be involved in the pro-inflammatory responses in AD. In this study, we investigated the role of FPRL1 in cellular uptake and the subsequent fibrillar formation of A β 42 by using fluorescence confocal microscopy. We found that upon incubation with macrophages or HEK293 cells genetically engineered to express FPRL1, A β 42 was associated with FPRL1 and the A β 42/FPRL1 complexes were rapidly internalized into the cytoplasmic compartment. The maximal internalization of A β 42/FPRL1 complexes occurred by 30 min after incubation. Removal of free A β 42 from culture supernatants at 30 min resulted in a progressive recycling of FPRL1 to the cell surface and degradation of the internalized A β 42. However, persistent exposure of the cells to A β 42 over 24 h resulted in retention of A β 42/FPRL1 complexes in the cytoplasmic compartment and the formation of Congo-red positive fibrils in macrophages, but not in HEK 293 cell transfected with FPRL1. These results suggest that in addition to mediating the pro-inflammatory activity of A β 42, FPRL1 is also involved in the internalization of A β 42, which culminates in the formation of fibrils only in macrophages.

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IDENTIFICATION OF NPI-1302a-3, AN ORALLY ACTIVE TNF AND IL-1 SYNTHESIS INHIBITOR WITH A NOVEL MECHANISM OF ACTION

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Protein based anti-TNF therapies are highly efficacious in rheumatoid arthritis

and inflammatory bowel disease yet these therapies are not effective in all patients and do not fully control disease progression. Inhibition of both TNF and IL-1 should more effectively regulate inflammation and cartilage degradation. Our studies were undertaken to characterize the bioactivities of novel TNF and IL-1 inhibitors. Analogs of an orally active multicyclic natural small molecule (< 400MW) were screened for their ability to inhibit cytokine production. One analog, NPI-1302a-3, exhibited low ng/ml activity for inhibiting TNF and IL-1 synthesis. Pretreatment of RAW-264.7 and THP-1 cells with 1 ug/ml of NPI-1302a-3 resulted in inhibition of TNF synthesis by approximately 75, IL-1 by 65 and IL-6 by only 30 percent. The synthesis of IL-8, IL-12 IL-1ra or IL-10 as well as cAMP levels were not significantly affected, suggesting mechanisms distinct from known p38 kinase and phosphodiesterase IV inhibitors. In addition, NPI-1302a-3 inhibited a specific cytokine signaling pathway in LPS-stimulated rat cardiomyocytes and other cell types. Orally, NPI-1302a-3 showed significant bioavailability and markedly inhibited TNF synthesis in LPS-challenged mice. The data suggest NPI-1302a-3 is a novel oral TNF and IL-1 synthesis inhibitor with a unique mechanism of action for regulating cytokine gene expression and signal transduction. The use of NPI-1302a-3 as a therapy for cytokine-mediated diseases may provide significant advantages over the current injectable drugs.

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Pulmonary inflammation induced by *Pseudomonas aeruginosa* virulence factors: role of IRF-1

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Chronic pulmonary infection with *P. aeruginosa* accounts for most of the morbidity in cystic fibrosis (CF) patients. The *P. aeruginosa* virulence factors LPS, phospholipase C (PLC) and exotoxin A (ETA) were evaluated for their ability to induce pulmonary inflammation in mice following intranasal inoculation. Both LPS and PLC induced high levels of TNF α , IL-1 β , IL-6 and the chemokines MIP-1 α and MIP-2 in the lungs, whereas IL-18 levels were not affected. ETA did not induce TNF α and was a weak inducer of IL-1 β , IL-6, MIP-1 α and MIP-2. In contrast, ETA markedly reduced constitutive lung IL-18 levels. LPS was the only virulence factor inducing IFN γ . LPS, PLC and ETA all induced cell infiltration as evaluated by pulmonary myeloperoxidase (MPO) activity and cell counts in the bronchoalveolar lavage fluid. Because levels of the IRF-1 are reduced in the nasal epithelium of mice with CF, the role of IRF-1 in pulmonary inflammation induced by LPS, PLC and ETA was evaluated. When inoculated with LPS, IRF-1 KO mice produced significantly lower levels of TNF α , IL-1 β and IFN γ . Similarly, a milder effect of ETA on IL-1 β and IL-18 was observed in IRF-1 KO compared to WT mice. On the contrary, the cytokine response to PLC did not differ between WT and IRF-1 KO mice. Despite differences in cytokine production, IRF-1 deficiency had no effect on MIP-1 α and MIP-2 levels and on MPO activity induced by LPS or ETA. Flow cytometric evaluation of lung mononuclear cells revealed strongly reduced percentages of CD8⁺ and NK cells in IRF-1 KO compared to WT mice. These data indicate that different virulence factors from *P. aeruginosa* induce pulmonary inflammation *in vivo* and that IRF-1 is involved in some of the cytokine responses to LPS and ETA. Supported by the Cystic Fibrosis Foundation.

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Protective Effects of IL-18 during the Acute Phase of Colitis: Potential Role for IL-11

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The intestinal epithelium is an active participant in establishing and maintaining gut homeostasis and produces a variety of cytokines in response to activation or injury. Intestinal epithelial cells (IEC) are a major source of IL-18, which is strongly upregulated in IBD. The aim of the present study was to investigate, *in vivo*, the role of IL-18 in mediating mucosal immune responses in an acute model of DSS colitis. 5% DSS was administered to IL-18 KO and

WT mice for 5d followed by a 7d recovery period. Water intake, hemocult status and weight were monitored daily, and colonic tissues collected for histologic and cytokine analyses. 50% mortality was observed in IL-18 KO mice compared to 0% in WT. Histologic assessment showed areas of active epithelial regeneration and restitution, with increased inflammation (3.7 ± 0.8 vs 0.8 ± 0.3 , $p < 0.02$) and areas of ulcerations (2.7 ± 0.8 vs 0.3 ± 0.2 , $p < 0.03$) in IL-18 KO vs WT mice. In WT, IL-18+ cells were localized to IEC with minimal staining in the lamina propria. IL-11, (known to protect clonogenic stem cells, regulate IEC proliferation and inhibit IEC apoptosis), was intensely detected in IEC from WT, and absent in IL-18 KO mice. In vitro studies performed using the I407 cell line supported IL-18's ability to potentially induce IL-11 from IEC in a time and dose-dependent manner. Our results suggest that IL-18 has a protective function in the acute phase of mucosal immune responses to gut epithelial damage/injury, and this effect may be dependent on IL-11; these data implicate a differential role of IL-18 in acute vs chronic phases of gut inflammation.

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Co-dependency of Tumor Regression and Anti-angiogenesis on both IFN- γ and Fas/FasL in Mice Treated with IL-12/pulse IL-2

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Novel in vivo assays of tumor neovascularization and electron microscopic analyses have allowed us to demonstrate that CD8⁺ T cell-dependent tumor regression induced by IL-12/pulse IL-2 is preceded by anti-angiogenic effects in the tumor. These effects coincide with rapid recruitment of CD8⁺ T cells, enhanced cell surface FasL expression on CD8⁺ T cells, and IFN- γ -dependent induction of both Fas and FasL gene expression within the tumor microenvironment. Inhibition of tumor neovascularization and induction of tumor regression are both critically-dependent on endogenous IFN- γ production and an intact Fas/FasL pathway. Most interestingly, rapid destruction of tumor-associated endothelial cells is detected in mice after treatment with IL-12/pulse IL-2, and this effect as well as regression of established metastatic tumors is ablated in mice with a dysregulated Fas pathway. The implication of a common critical role for endogenous IFN- γ and the Fas/FasL pathway in early anti-vascular and anti-angiogenic effects, as well as subsequent complete tumor response, suggests a unique interdependence between several rapidly engaged cytokine-driven innate immune mechanisms and CD8⁺ T cell-dependent responses.

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DEFECTIVE NF- κ B ACTIVATION IN TUMOR ASSOCIATED MACROPHAGES

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We have previously reported that tumor associated macrophages (TAM) show defective IL-12 gene expression, a central cytokine in the activation of inflammation and immunity and in the generation of Th1-type responses. This immunosuppressed phenotype was paralleled by increased production of the inhibitory cytokine IL-10 and defective NF- κ B activation. In the attempt to elucidate the molecular mechanisms responsible for defective expression of NF- κ B dependent genes, we investigated the pathway of NF- κ B activation following cells treatment with LPS (100 ng/ml), both in TAM and peritoneal macrophages (PEC). In TAM, defective activation of the p50/p65 NF- κ B heterodimer was associated with a massive nuclear localization of the inhibitory p50 NF- κ B protein (5-10 fold higher compared to PEC). Moreover, in contrast with PEC, TAM displayed impaired nuclear translocation of the transactivating p65 NF- κ B member. The inhibition of the LPS-dependent nuclear translocation of NF- κ B p65 in TAM was substantiated by the lower level of protein expression of the LPS and interleukin-1 (IL-1) receptor-associated kinase (IRAK). This molecular pathway most likely underlies other

alterations of TAM functions dependent on NF- κ B activation and represents a severe impairment for inflammatory and immune responses. Our study points to the NF- κ B system as a major target of the immunosuppressive mechanisms elicited by tumors on macrophage functions. Presenting author: Dr. Antonio Sica Istituto di Ricerche Farmacologiche Mario Negri via Eritrea 62, 20157 Milan, ITALY tel: 0239014530 fax: 0233200231 email: sica@ifrmm.mnegr.it

ADAPTIVE IMMUNITY (355-362)

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Contrasting roles of IL-2 and IL-15 in the life and death of lymphocytes: implications for immunotherapy

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Although IL-2 and IL-15 share two receptor subunits and many functions, especially in innate immunity, they provide distinct and at times contrasting contributions to adaptive immune responses. IL-2 is pivotally involved in AICD. In contrast, studies in our IL-15 transgenic mice indicate that IL-15 inhibits this process. Furthermore, IL-15 stimulates the development of CD8⁺ memory phenotype T-cells that are elevated in IL-15 transgenic mice. IL-2 inhibits this process. In particular, the addition of antibodies to IL-15 or its receptor inhibits the ex vivo survival of antigen specific MHC restricted CD8 cells from patients with HTLV-I associated tropical spastic paraparesis. The opposing effects of IL-2 and IL-15 on AICD and memory CD8 cells have implications for the cytokine directed treatment of autoimmune diseases and cancer. We are developing IL-15 for the treatment of cancer and as a component of vaccines and have prepared a humanized antibody to the IL-15R beta subunit that blocks IL-15 action for the treatment of autoimmune diseases and HTLV-I associated disorders.

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The B7RP-1/ICOS T-cell Co-stimulation Pathway

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The co-stimulatory proteins in the CD28- and B7-related protein families are involved in the regulation of the immune response. We have found a receptor-ligand pair in a novel T-cell co-stimulatory pathway. The CD28-related protein is called ICOS, for inducible co-stimulator, and the B7-related protein is termed B7RP-1. These genes were discovered in a cDNA library from murine intestinal intraepithelial lymphocytes. B7RP-1 enhances an immune response. Expression of the B7RP-1-Fc fusion protein in transgenic mice results in lymphoid hyperplasia and an intestinal defect similar to human Crohn's disease. These B7RP-1-Fc expressing transgenic mice also have increased IgG1, IgG2a, and IgE levels. Conversely, mice lacking the ICOS gene have decreased IgG1, IgG2a, and IgE levels and demonstrate an impaired immune response. These and other experiments indicate that B7RP-1 is the ligand for a positive co-stimulatory pathway that is involved in the late events of a primary T-cell response, or in a memory response. Syngeneic mouse tumor models have demonstrated robust B7RP-1-Fc anti-tumor activities. B7RP-1-Fc works primarily on immunogenic tumors, such as sarcomas. We show that B7RP-1-Fc effectively causes the rejection of Meth A, SA-1, and EMT6 tumors in the syngeneic mouse hosts. Established Meth A tumors were rejected effectively with either four doses of 0.3 mg/kg, or a single dose of 1 mg/kg, beginning on day 7. Delayed B7RP-1-Fc treatments that were initiated on day 17 or 24, instead of day 7, were less effective. Mice that previously rejected a MethA tumor also rejected a subsequent challenge on day 60 by Meth A without further treatment, indicating a long-lived memory response. Thus, the administration of soluble B7RP-1-Fc may have therapeutic value in generating or enhancing anti-tumor activity in a clinical setting. The CD28-regulated pathway is involved in co-stimulation of resting or naïve T-cells. The ICOS regulated pathway appears to act at a later stage in T-cell activation, perhaps having a functional role in secondary T-cell responses. Here, we

characterize the B7RP-1/ICOS pathway and determine the therapeutic opportunities in targeting this pathway.

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IL-7 and IL-15 are essential for the proliferation and survival of memory CD8 T cells

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IL-7 and IL-15 are cytokines that play important roles in immune system development and function. IL-7 has been shown to be important for the development, proliferation and survival of naive and memory CD4 and CD8 T cells. IL-15 on the other hand is important for the development of NK cells and has been implicated as an important factor for memory CD8 but not memory CD4 T cells. Using IL-15 KO mice and an antibody directed against the IL-7R α chain, we report here studies that dissect the roles of these cytokines for memory CD8 T cell proliferation and survival. IL-7 but not IL-15 is required for the acute homeostasis driven proliferation (HDP) of naive CD8 T cells (OTI Tg T cells as well as polyclonal CD44lo) in irradiated hosts. In contrast, acute HDP of memory CD8 T cells (OTI or polyclonal CD44hi) is delayed in IL-15 KO mice or by treatment of wild type mice with anti-IL-7R α mAb. In the absence of IL-15 and inhibition of IL-7R α function, proliferation is almost completely inhibited. Furthermore, basal homeostatic proliferation of CD8 memory T cells in a full T cell compartment is blocked in IL-15 KO mice and delayed by treatment with anti-IL-7R α mAb in wild type mice. In the absence of IL-15 and inhibition of IL-7R function, survival of cells is substantially decreased. These results suggest that IL-7 and IL-15 are essential for the proliferation and survival of memory CD8 T cells.

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Leptin: A pivotal mediator for intestinal inflammation

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Leptin the product of the *ob* gene, regulates the balance of Th1/Th2 cytokines and modifies T cell immunity. To study the influence of leptin in autoimmune diseases such as inflammatory bowel disease (IBD), either acute or chronic colitis was induced in leptin-deficient *ob/ob* wild type (WT) mice using dextran sulfate sodium (DSS) in the drinking water. *Ob/ob* mice showed an over 70% reduction of colitis severity in the acute model (weight loss, stool consistency and bleeding) and colon shortening compared to WT mice. Spontaneous production of IL-18, IFN γ , TNF α and IL-1 β was significantly suppressed in colon cultures. Leptin replacement converted disease resistance to susceptibility indicating that leptin deficiency is responsible for resistance to acute DSS-induced colitis. These experiments were confirmed in the model of chronic colitis where macroscopic and histologic parameters showed an over 70% reduction of colitis severity in the *ob/ob* mice. This was accompanied by a reduced production of TNF α , IL-1 β , IL-6, IFN γ , IL-18 and the chemokines MIP-1 α and MIP-2 and a decreased neutrophilic infiltration in the colon. Characterization of intestinal lymphocytes revealed a 20% reduction of CD8+ intraepithelial lymphocytes. Furthermore, *ob/ob* CD8+ cells showed a 90% reduction of IFN γ synthesis compared to WT cells. Activation of the leptin receptor results in STAT-3 phosphorylation. In colonic tissue of DSS-exposed *ob/ob* mice, no phosphorylated STAT-3 was detectable, whereby in WT mice STAT-3 was strongly activated. In conclusion, these results demonstrate that leptin represents a functional link between the endocrine and the immune system, which requires further investigations in experimental models and human IBD.

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The TNFR family member HVEM plays a role in thymic selection processes

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The Herpes-virus-entry-mediator (HVEM) is a recently identified TNF Receptor family member thought to be involved in T cell activation. In addition to prominent expression on peripheral T cells, we found HVEM is present on CD4/CD8 double-positive (DP) thymocytes, a stage in thymic development where selection occurs. The present study was undertaken to investigate the role of HVEM in T cell maturation. To mimic signaling through HVEM during T-cell development in vitro, anti-HVEM antibodies or murine LIGHT, a natural ligand of HVEM, were introduced into a fetal thymic organ culture system (FTOC). After 10 days of culture the thymic cultures were analyzed for cell viability and differentiation of T-cell subsets. We find that engagement of HVEM with antibody results in a decrease in DP thymocytes and a selective increase in CD4 SP thymocytes. Interestingly, the addition of mLIGHT to the culture induces comparable effects, suggesting that HVEM may promote negative selection events. The TCR Vb8-specific superantigen SEB was used to induce negative selection in FTOC. Vb8-expressing cells could be rescued from deletion by addition of soluble decoy receptors of HVEM or Lymphotoxin-b receptor, which also binds to LIGHT. Thus, expression of LIGHT in the thymus may also contribute to positive selection. Together, our data suggest a prominent role for the HVEM/LIGHT receptor ligand pair in thymic selection. This work is supported in part by NIH grants AI33068, CA69381 and AI48073.

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Overexpression of murine IL-17E induces a Th 2-like response and multi-organ inflammation in transgenic mice

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IL-17E is a recently described member of an emerging family of IL-17 related cytokines. IL-17E has been shown to bind IL-17Rh1, a protein distantly related to the receptor for IL-17, suggesting that IL-17E likely possesses unique biological functions. Here we have identified the murine ortholog of IL-17E and developed transgenic mice in order to characterize the actions of this new cytokine. Overexpression of IL-17E resulted in eosinophilia, and increased serum IgE and IgG1, but not IgG2a, suggesting a Th 2-like immunologic response. Consistent with this, serum levels of IL-13 and IL-5 were also elevated in the transgenic mice. Elevated gene expression of several Th2 cytokines, including IL-4, IL-5, IL-10 and IL-13 was observed in multiple tissues. Albeit a systemic Th 2-like response, tissue-specific expression patterns of cytokines, chemokines and adhesion molecules, and other immunologic changes were also seen. Moreover, exposure to IL-17E induced pathological changes in multiple tissues, particularly liver, heart, and lungs, characterized by mixed inflammatory cell infiltration, epithelial hyperplasia and hypertrophy. These findings suggest that IL-17E is a unique pleiotropic cytokine that engages a systemic Th 2-like response with tissue-specific immunological and pathological changes.

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The regulatory effect of epithelial production of transgenic IL-10 on mucosal immune responses

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To explore the effect of a site-specific delivery of interleukin (IL)-10 on intestinal immune responses, we have created transgenic mice in which IL-10 is expressed by the intestinal epithelium. Transgenic mice showed a marked increase in the number of intraepithelial lymphocytes (IEL) in the small intestine. Mucosal lymphocytes of transgenic animals produced less T helper (Th) 1 cytokines than wildtype lymphocytes. By contrast, the production of transforming growth factor (TGF)- β was increased. Moreover, the epithelial layer in transgenic mice was significantly enriched for CD4CD25 T cells. Collectively the data suggest that epithelial IL-10 may act by increasing the number or activity of regulatory T cells. Furthermore, transgenic mice had increased production of immunoglobulin (Ig) A in the small intestine. These effects were local, as splenic lymphocytes were not affected. Studies in models of inflammatory bowel disease (IBD) demonstrated that transgenic IL-10 was able to attenuate the chronic intestinal inflammation arising spontaneously in IL-10 $^{-/-}$ mice, as well as the acute colitis induced by dextran sodium sulfate (DSS) administration or by adoptive transfer of CD4CD45RB^{high} splenocytes. These observations provide evidence for an *in vivo* lympho-epithelial crosstalk, by which locally produced IL-10 can regulate immune responses in the intestine, without systemic modifications.

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TRANSFORMING GROWTH FACTOR- β 1 INDUCES ANERGIC/ SUPPRESSOR CD4⁺ CD25⁺ CTLA-4⁺ T CELLS

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Evidence has emerged that a subset of CD4⁺ T cells function as anergic/ suppressor T cells. Whether these anergic/suppressor CD4⁺ T cells develop from a defined lineage or represent a stage that multiple types of CD4⁺ T cells can acquire remains unclear. To define the derivation of this regulatory cell population, we added TGF- β to splenic CD4⁺ T cells and examined the phenotypic and functional consequences. We present evidence that TGF- β induces anergic/suppressor CD4⁺ T cells, which express CD25, intracellular CTLA-4 and are CD45RB^{-low}. These anergic/suppressor cells were anergic specifically to TCR stimulation, produced neither Th1 nor Th2 cytokines, but secreted TGF- β and inhibited normal T cell activation *in vitro* and *in vivo*. IL-2 abrogated the role of TGF- β in driving the anergic/suppressor phenotype. TGF- β preserved intracellular CTLA-4 of CD4⁺ T cells by preventing its degradation through the inhibition of transcription factor AP-1 expression and thus, AP-1/CTLA-4 complex formation. Significantly, TGF- β also converts proliferative CD4⁺CD25⁻CTLA-4⁻ T cells into anergic CD4⁺CD25⁺CTLA-4⁺ cells. The data provide insight into understanding the mechanisms for generation and development of CD4⁺ anergic/suppressor T cells, which has implications for T cell tolerance and T cell-based immunotherapy.

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NATIONAL MEETINGS

- 1st **December 14-16, 1964.** New York, NY
N.R. DiLuzio and F.J. DiCarlo, Chairs
Abstracts: *J. Reticuloendothel. Soc.* (1964) 1:343-368
- 2nd **December 8-11, 1965.** Salt Lake City, UT
T.F. Dougherty and D.L. Berliner, Chairs
Abstracts: *J. Reticuloendothel. Soc.* (1965) 2:343-364
- 3rd **November 28-30, 1966.** Bethesda, MD
M. Landy, Chair
Abstracts: *J. Reticuloendothel. Soc.* (1966) 3:250-382
- 4th **December 3-6, 1967.** Winston Salem, NC
Q.N. Myrvik, Chair
Abstracts: *J. Reticuloendothel. Soc.* (1967) 4:419-455
- 5th **December 1-4 1968.** New York, NY
F.J. DiCarlo, Chair
Abstracts: *J. Reticuloendothel. Soc.* (1968) 5:550-598
- 6th **December 2-5, 1969.** San Francisco, CA
E.L. Dobson, Chair
Abstracts: *J. Reticuloendothel. Soc.* (1970) 7:627-666
- 7th **December 2-5, 1970.** Augusta, GA
S.M. Reichard, Chair
Abstracts: *J. Reticuloendothel. Soc.* (1971) 9:592-647
- 8th **November 30-December 3, 1971.** Detroit, MI
J. Rebuck, Chair
Abstracts: *J. Reticuloendothel. Soc.* (1972) 11:394-440
- 9th **December 5-8, 1972.** Austin, TX
L.J. Berry, Chair
Abstracts: *J. Reticuloendothel. Soc.* (1973) 13:343-395
- 10th **December 5-8, 1973.** Williamsburg, VA
W. Regelson and W.R. Wooles, Chairs
Abstracts: *J. Reticuloendothel. Soc.* (1974) 15:1a-84a
- 11th **December 2-5, 1974.** Seattle, WA
N.B. Everett, Chair
Abstracts: *J. Reticuloendothel. Soc.* (1974) 16:1a-57a
- 12th **December 4-8, 1975.** Miami, FL
M.M. Sigel, Chair
Abstracts: *J. Reticuloendothel. Soc.* (1975) 18:1b-55b
- 13th **December 15-18, 1976.** New Orleans, LA
N.R. Diluzio, Chair
Abstracts: *J. Reticuloendothel. Soc.* (1976) 20:1a-66a
- 14th **December 6-9, 1977.** Tuscon, AZ
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- 15th **December 6-9, 1978.** Charleston, SC
H.H. Fudenberg, Chair
Abstracts: *J. Reticuloendothel. Soc.* (1978) 24:1a-71a
- 16th **December 5-8, 1979.** San Antonio, TX
D.E. Thor, Chair
Abstracts: *J. Reticuloendothel. Soc.* (1979) 26:1a-58a
- 17th **December 2-5, 1980.** Tampa, FL
- 18th **October 13-16, 1981.** Milwaukee, WI
P. Abramoff, Chair
- 19th **October 17-20, 1982.**
S.D. Douglas, Chair
Abstracts: *J. Reticuloendothel. Soc.* (1982) 32:49-85
- 20th **October 9-12, 1983.** Portland, OR
R.I. Mishell, Chair
Abstracts: *J. Reticuloendothel. Soc.* (1983) 34:153-193
- 21st **October 14-17, 1984.** Montreal, Canada
D.O. Adams, Chair
Abstracts: *J. Leukoc. Biol.* (1984) 35:179-257
- 22nd **August 3-8, 1985.** Ithaca, NY
Joint Conference of the 17th International Leukocyte Culture and 22nd National Meeting of the Reticuloendothelial Society
P.A. Campbell, D.M. Jacobs, J.J. Oppenheim, Chairs
Abstracts: *J. Leukoc. Biol.* (1985) 38:47-190
- 23rd **September 28-October 1, 1986.** Denver, CO
P. Ralph, Chair
Abstracts: *J. Leukoc. Biol.* (1986) 40:221-332
- 24th **October 17-21, 1987.** Kauai, HI
T.S. Edgington, G. Poste, R.B. Herberman, Chairs
Abstracts: *J. Leukoc. Biol.* (1987) 42:279-441
- 25th **October 27-30, 1988.** Washington D.C.
P.M. Henson, Chair
Abstracts: *J. Leukoc. Biol.* (1988) 44:223-312
- 26th **October 12-15, 1989.** Marco Island, FL
T.A. Springer, Chair
Abstracts: *J. Leukoc. Biol.* (1989) 46:280-407
- 27th **October 14-18, 1990.** Heraklion, Crete, Greece
M. Meltzer and A. Mantovani, Chairs
Abstracts: *J. Leukoc. Biol.* (1990) Supplement 1:15-104
- 28th **September 28-October 1, 1991.** Snowmass-Aspen, CO
J. Cambier and P. Lipsky, Chairs
Abstracts: *J. Leukoc. Biol.* (1991) Supplement 2:16-110
- 29th **December 2-5, 1992.** Charleston, SC
R. Snyderman, Chair
Abstracts: *J. Leukoc. Biol.* (1992) Supplement 3:13-54
- 30th **September 21-24, 1994.** Tuscon, AZ
C. Nathan, Chair
Abstracts: *J. Leukoc. Biol.* (1994) Supplement: 17-39
- 31st **September 13-16, 1995.** Marco Island, FL
I. Fidler, Chair
Abstracts: *J. Leukoc. Biol.* (1995) Supplement: 7-32

ANNUAL MEETINGS

(Renamed by SLB Council)

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| <p>32nd December 3-7, 1997. Baltimore, MD
M.A.S. Moore, Chair
Abstracts: <i>J. Leukoc. Biol.</i> (1997) Supplement: 7-28</p> <p>33rd August 22-25, 1998. La Jolla, CA
G.M. Bokoch, Chair
Abstracts: <i>J. Leukoc. Biol.</i> (1998) Supplement 1:1-35</p> | <p>34th October 5-8, 2000. Cambridge, MA
C.A. Janeway, Jr., J. Stein-Streilein, Chairs
Abstracts: <i>J. Leukoc. Biol.</i> (2000) Supplement: 1-100</p> <p>35th November 8-11, 2001. Maui, HI.
T. Hamilton, A. Mantovani, Chairs
Abstracts: <i>J. Leukoc. Biol.</i> (2001) Supplement</p> |
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INTERNATIONAL CONGRESSES

- | | |
|--|---|
| <p>1st July 4-8, 1955. Paris and Gif-Sur-Yvette, France
B.N. Halpern and C.A. Doan, Chairs
Proceedings: <i>Physiopathology of the Reticuloendothelial System</i>. B.N. Halpern (ed.) C.C. Thomas, Springfield</p> <p>2nd August 27- September 1, 1956. Boston, MA, USA
C.A. Doan, Chair
Abstracts: <i>Proceedings of the Sixth Congress of the International Society of Hematology</i>. Grune & Stratton, New York, 1958</p> <p>3rd August 28-31, 1958. Rapallo, Italy
A.M. Marmont, Chair
Proceedings: <i>Reticuloendothelial Structure and Function</i>. J.H. Heller (ed.) The Ronald Press Co., New York, 1960</p> <p>4th May 29-June 1, 1965. Otsu and Kyoto, Japan
G. Wakisaka, Chair
Proceedings: <i>Reticuloendothelial System: Morphology, Immunology and Regulation</i>. Nissha Co., Kyoto, 1965</p> <p>5th September 8-10, 1966. Como, Italy
R. Paoletti, Chair
Proceedings: <i>The Reticuloendothelial System and Atherosclerosis</i>. N.R. DiLuzio and R. Paoletti (ed.) Plenum Press, New York, 1967</p> <p>6th July 29-August 1, 1970. Friburg, Germany
K.B.P. Flemming, Chair
Proceedings: <i>The Reticuloendothelial System and Immune Phenomena</i>. N.R. Diluzio and K. Flemming (ed.) Plenum Press, New York, 1971</p> <p>7th July 1975. Pamplona, Spain
A. Oehling, Host
Abstracts: <i>J. Reticuloendothel. Soc.</i> (1975) 18:1a-41a
Proceedings: <i>The Reticuloendothelial System in Health and Disease</i>. Part A- Functions and Characteristics; Part B- Immunologic and Pathologic Aspects. S.M. Reichard, M.R. Escobar and H. Friedman (eds.) Plenum Press, New York, 1976</p> | <p>8th June 18-23, 1978. Jerusalem, Israel
M. Schlesinger and M.M. Sigel, Chairs
Abstracts: <i>J. Reticuloendothelial Soc.</i> (1978) 23:11-541
Proceedings: <i>Macrophages and Lymphocytes: Nature, Functions and Interactions</i>. M.R. Escobar and H. Friedman (eds.) Plenum Press, New York, 1979</p> <p>9th February 8-12, 1982. Davos, Switzerland
E. Sorkin, D. Wilkins and S. Normann, Chairs
Proceedings: <i>Macrophages and Natural Killer Cells. Regulation and Function</i>. S.J. Normann and E. Sorkin (eds.) Plenum Press, New York, 1982</p> <p>10th September 2-7, 1984. Ito, Japan
D.O. Adams and R. Snyderman, Chairs
Abstracts: <i>J. Leukoc. Biol.</i> (1984) 36:179-257
Proceedings: <i>Macrophage Biology</i>. S.M. Reichard and M. Kojima (eds.) Alan R. Liss, New York, 1985</p> <p>11th October 17-21, 1987. Kauai, HI
T.S. Edgington, G. Poste, and R.B. Herberman, Chairs
Abstracts: <i>J. Leukoc. Biol.</i> (1987) 42:279-441</p> <p>12th October 14-18, 1990. Heraklion, Crete, Greece
M. Meltzer and A. Mantovani, Chairs
Abstracts: <i>J. Leukoc. Biol.</i> (1990) Supplement 1:15-104</p> <p>13th December 1-5, 1993. Sydney, Australia
<i>Regulation of Leukocyte Production and Immune Function</i>
E.R. Stanley, Chair
Abstracts: <i>J. Leukoc. Biol.</i> (1993) Supplement: 33-139</p> <p>14th October 11-14, 1996. Verona, Italy
<i>The Phagocyte: Molecular and Clinical Aspects</i>
J.S. Haskill and R. Andreesen, Chairs
Abstracts: <i>J. Leukoc. Biol.</i> (1996) Supplement: 13-66</p> <p>15th September 22-26, 1999. Cambridge, UK
<i>Innate Resistance to Infection</i>
P. Scott, J.M. Blackwell, B. Zwillig, Chairs
Abstracts: <i>J. Leukoc. Biol.</i> (1999) Supplement: 9-34</p> |
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CONSTITUTION

ARTICLE I (Name)

The name of the society shall be the SOCIETY FOR LEUKOCYTE BIOLOGY.

ARTICLE II (Purpose)

The purpose of the Society shall be:

1. To promote original research in the fields of leukocyte biology and host defense.
2. To provide a forum for the multidisciplinary integration of current basic and clinical knowledge and concepts in the fields of leukocyte biology and host defense.
1. To promote the dissemination and applications of knowledge of these fields, including publishing an appropriate journal(s).
2. To promote an awareness of the national and international health importance of these fields.
5. To engage exclusively in educational and scientific activities in studies of leukocyte biology, including for such purposes the making of distributions only to organizations that qualify as exempt organizations under Section 501 (c) (3) of the Internal Revenue Code (or the corresponding provisions of any future United States Internal revenue law).

ARTICLE III (Membership)

Membership in the Society shall be open to persons who share the stated purpose of the Society and who have educational, research, or clinical interest in the field.

ARTICLE IV (Officers)

The officers of the Society shall be a President, a President-elect, a Secretary, and a Treasurer. The President-elect shall serve one year as such, followed by one year as President. No person shall ever be eligible for re-election to the Presidency.

The Secretary and Treasurer shall be elected to a term of two years. The Secretary and the Treasurer shall be elected biennially and may serve two terms. Election shall be by secret ballot. The terms of office shall begin January 1 following the annual meeting at which they were elected.

ARTICLE V (Council)

There shall be a Council responsible for the fulfillment of the scientific and business obligations of the Society.

The current officers, the immediate Past-President, the Editor-in-Chief of the official Society journal(s), and eight additional Councilors shall constitute this Council. Councilors shall be elected to provide representation from the various areas of leukocyte biology research. Councilors shall be chosen by the membership of the Society for four-year terms, two to be elected each year.

ARTICLE VI (Affiliations)

The Society is empowered to affiliate with other organizations. Proposals for affiliation may be initiated by individual Members of the Council or by a petition of the Council signed by ten Members of the Society. To become effective petition must be approved by a two-thirds majority of the Council and approved by the membership.

ARTICLE VII (Bylaws)

The provisions of the Constitution of the Society shall be carried out in accordance with the current Bylaws of the Society.

ARTICLE VIII (Amendments)

Amendments may be initiated by individual Members of the Council or by a petition to the Council signed by ten Members of the Society. Amendments must be approved by a two-third's majority of the Council and be ratified in a mail ballot by majority of those Members of the Society voting.

ARTICLE IX (Finances)

All fiscal affairs of the Society shall be conducted on the basis of the Calendar year. Statements of assets, income, expenditures and capital funds shall be audited annually by an independent auditing firm. A financial statement of the assets of the Society shall be published annually. Persons having signatory powers for the funds of the Society shall be designated by Council and shall be bonded.

ARTICLE X (Divestiture)

It is intended that the existence of the Society shall be perpetual. However, should the Society be terminated for any reason, the residual funds of the Society shall be assigned to one or more not-for-profit organizations engaged in activities similar to those of the Society for Leukocyte Biology and qualified as an exempt organization under Section 501 (C) (3) of the 1954 internal Revenue Code.

BYLAWS

ARTICLE I (Membership)

- (1) The membership of the Society shall consist of Members, Student Members, Associate Members, Emeritus Members, Honorary Life Members, and Corporate Members. Applications must be approved by the Membership Committee.
- (2) **Members.** A person who shares the stated purpose of the Council and is eligible under Article III of the Constitution may be elected a Member. An active member may participate in the scientific and business sessions of the Society and is eligible for election to office. There will be no restrictions because of place of birth, residence, sex, race, age or creed.
- (3) **Student Members.** The principal requirement for Student Membership is a genuine and active interest in the aims and purposes of the Society. Applicants must be sponsored by an active member of the Society. The fee for Student Membership shall be the Society's cost of the Journal, or 1/2 of the Society's dues without the Journal. Membership shall be renewable each year for as long as the individual is a full-time student. Application for Full Membership in the Society is then required. Student Membership does not include voting privileges in the Society.
- (4) **Emeritus Members.** A Member in good standing for 10 years, who has retired or attained the age of 65 may apply to the Council for election to emeritus status. Emeritus Members shall pay no dues but shall have all rights and privileges of Members. They are eligible to receive the journal at the reduced fee granted to active members.
- (5) **Honorary Life Members.** Two individuals may be recommended by the Council annually to Honorary Life Membership as a tribute to their contributions to the knowledge of leukocyte biology. Such nominees are to be elected by two-thirds of the membership attending the annual business meeting. Such members shall be exempt from Society dues and the annual meeting registration fee. They shall possess all rights and privileges of active members and shall receive the journal free of charge.
- (6) **Corporate Members.** An association, corporation, or institution desiring to support the Society may be invited to become a corporate member.

ARTICLE II (Meeting)

The Society is authorized to hold scientific meetings, international, national, and regional. A business meeting shall be held in connection with the annual scientific meeting of the Society. Parliamentary procedures to be followed in the business meeting shall be those specified in "Robert's Rules of Order." Five percent of the Members, or 50, whichever is smaller, shall constitute a quorum.

ARTICLE III (Dues)

Membership dues may be changed by the Council, subject to approval at the next Annual Business Meeting. Annual dues are payable on December 1st preceding the beginning of the fiscal year. Members who have not paid by January 1st will be notified every six months for one year and then dropped from the mailing list. A member may be reinstated with full seniority upon payment of past dues.

ARTICLE IV (Publications)

The Society is empowered to publish or to enter into agreements with others to publish such journals and other publications (abstracts, review, newsletters etc.) as may be authorized by a two-thirds majority vote of the Council. Change in the agreements which implement the publishing of a duly established journal or other organ may be authorized by a majority vote of the Council. An Editor-in-Chief shall be elected from the membership by a majority vote of the Council to serve for four years. He shall appoint, with the consent of the council an Editorial Board and Assistant Board and Assistant Editors as needed. These Assistant Editors are to be chosen because of their ability in specialized fields. The Editor-in-Chief shall make a report of his stewardship of the Journal at the annual meeting. His report shall summarize the editorial situation and include the number of manuscripts received, rejected, accepted, and published during the year; changes in editorial personnel; a summary of circulation and of finances and any other information that the Editorial Board may feel to be pertinent or which may be required by the Council.

ARTICLE V (Duties of Officers)

It shall be the duty of the President to preside over the annual business meeting of the Society, to serve as Chair of the Council, to appoint and charge, with the approval of the Council, the Chair and members of all committees of the Council, and to carry out other activities usually pertaining to the office. The President-elect shall carry out the duties of an absent or disabled President. The President-elect will automatically succeed to the presidency when the office becomes vacant.

The Secretary shall keep accurate records, maintain an up-to-date membership list, and give notice of all meetings of members and of the Council.

The Treasurer shall send out dues notices and collect all dues. S/he shall be responsible for all funds and securities of the Society, and shall make all disbursements in accordance with the budget approved by the Council. S/he shall submit an annual report of the financial condition of the Society and be responsible for any financial reports required by the Internal Revenue Service.

BYLAWS (continued)

ARTICLE VI (Duties of the Council)

The duties of the Council shall be to determine the policies for the good of the Society and the science it represents in accordance with the Constitution and to implement the execution of these policies as provided in these Bylaws. It shall plan the scientific meeting: it shall authorize the expenditure of Society funds, and it shall obtain an annual audit of the Society finances.

The Council shall appoint officers from councilors or councilors from the membership to fill vacancies that arise. Such appointees shall serve until the next regularly elected person takes office.

The Council may appoint an Administrator or Executive Director with appropriate compensation to assist in handling the affairs of the Society.

The Council shall meet at the call of the President, at least once a year. At the regular meeting it shall consider changes in dues, amendments to the Constitution and Bylaws, and proposals for affiliation, and set the agenda for the business meeting. Newly elected Council members who have not yet taken office, are expected to attend this meeting, but may not vote.

The Council shall have power to conduct other business by means of mail vote.

Six voting Members of the Council shall constitute a quorum. The Council may apply for grants or secure donations for specific projects which are consistent with the purposes of the Society. They or appropriate Committees of the Council may then meet to consider their business at times other than the Annual Meeting. Expenses may be defrayed by the Society as determined by the Council. The Council shall produce and distribute by January 15th each year a handbook that defines the duties of each officers, councilor and committee.

ARTICLE VII (Election)

Nominations for offices to become vacant shall be made by the Nominating Committee. Nominations will also be received by petition. Each petition must be signed by ten Members and must contain a written statement by the nominee of willingness to serve. In order that the names of persons so nominated may appear on the ballot, petitions must be received by the Secretary before January 1st. The final list of nominees arranged as a ballot, and containing more than one name for each vacancy to be filled, shall be mailed to the Members. The candidate for each office receiving the highest number of votes will be elected.

ARTICLE VIII (Standing Committees)

(1) Awards and Honors Committee. The Awards and Honors Committee shall normally be composed of the three Past-Presidents of the Society. Each President appoints one member to a three-year term and designates the Chair of the Committee. The Committee is charged with the responsibility for selecting finalists from the abstracts entered by students in training (Predoctoral or Postdoctoral). Finalists will present their work at the Annual Meeting. The Committee may also be charged with selecting a member of the Society who has shown consistent excellence in research. The award will be a named award. Any recommendation for new awards and honors made by the Council or membership will be referred to this Committee for discussion and recommendation. This Committee may also initiate recommendations and other ideas for Awards and Honors appropriate to the goals and objectives of the Society.

(2) Corporate Resources. The Chair of the Corporate Resources Committee shall be appointed for a three-year term and shall be a member of the Finance Committee. The Chair, with the consent of the President, may appoint additional members to the Committee as needed. The Corporate Resources Committee is responsible for (1) coordinating Society activities affecting corporation, (2) soliciting corporate members, (3) recommending benefits for corporate members, (4) coordinating the solicitation of sponsors of workshops and symposia at the Annual Meeting, (5) improving communication between the private sector and the Society.

(3) Finance Committee. The Finance Committee shall be composed of the Treasurer as Chair, the Chair of the Corporate Resources Committee, the Chair of the Meetings Committee and the President-elect. The administrative officer of the Society serves as an ex-officio member of this Committee. The Committee shall prepare an annual Society budget and submit it for Council approval at the time of the Annual Meeting and prior to the start of the fiscal year. This budget shall include estimates of all income sources and appropriate estimates of expenditures for committees, officers, meetings, and publications. The Finance Committee shall consider and attempt to devise ways to increase the Society's income.

(4) International Relations Committee. The International Relations Committee shall be composed of four members, three to be elected by Council from among four nominees submitted by the President. Their terms of office shall be for three years, one being elected each year. Members of this Committee shall be the official delegates to any International Meeting and be responsible for the foreign activities of the Society. The chairman of this committee will be the immediate past Scientific Program Chair of the International Congress, and the term is to run from one International Congress to the other. The Chair will be a voting member of Council.

(5) Membership Committee. The Membership Committee shall be composed of three members, each serving a term of three years. The primary purposes of the Committee are to increase individual memberships in the Society and to review applications for membership. Applicants may be granted membership by the Committee.

(6) Nominating Committee. The Nominating Committee shall be composed of three members appointed by the President, each serving three years, one being appointed each year. The senior member of the Committee shall serve as Chair. Committee members

Bylaws (continued)

may not currently be from the same institution. The Nominating Committee shall submit nominations for the officers of President-elect, Councilors, Secretary, Treasurer, and Scientific Program Committee. It will be the responsibility of the Nominating Committee to

prepare lists of nominees from the members and to ascertain the willingness of each nominee to serve. The Committee transmits nominations to the Secretary at least six months prior to the Annual Meeting. Other names may be added to the Ballot upon petition in accordance with the procedures published in Article VII of the Bylaws. At least 3 months before the Annual Meeting, a Ballot containing the list of all nominees will be sent to the membership. For a member to be eligible for nomination for election office, he/she must be an active member in good standing for a minimum of two years.

(7) Publications Committee. The Publications Committee shall be composed of four members appointed by the President, each serving four years, one being appointed each year. The senior member will be the Chair. The Editor(s)-in-Chief of all publications shall serve in a non-voting capacity. The Committee formulates general policy concerning all publications and makes decisions concerning publications arising out of Annual and International Meetings, subject to review and approval by the Council. The Committee is responsible for nominating an Editor-in-Chief for Council approval. The Committee serves as a liaison between the membership and the Journal, offering advice and comment on general publication policy.

(8) Rules Committee. The Rules Committee shall be composed of four appointed members, three of whom shall serve for a term of three years, one being appointed each year by the President. A fourth member shall serve as Chair of this Committee for a term of one year, and may be reappointed by the President to this position. The Chair of the Committee becomes the Parliamentarian of the Society with such duties as may be set forth in the Bylaws or Rules of the Society. Questions relative to the interpretation of the Constitution shall be presented to the Rules Committee. The duties of this Committee shall be to provide information for the Council on matters relating to the Constitution of the Society, its Bylaws, and acts of the Annual Meeting; to interpret for the Council and Constitution, Bylaws, and acts of the Annual Meeting; to recommend to the Council the requirements for, and privileges and obligations of, the several classes of membership; and to consider from time to time, either on its own initiative or by reference from the Council or the Membership, proposed revisions of the Constitution and Bylaws.

(9) Scientific Program Committee. The Scientific Program Committee shall be composed of six members, three elected members and three members appointed by the elected members. Elected members shall each serve three years, one being elected each year, and shall Chair the Committee in their third year. Elected members shall be nominated by the Nominating Committee and these nominees should represent the scientific interests of the Society.

The Scientific Program Committee develops the program for the Annual Meeting, including topics and contributors for major sessions and selection of preferred papers. This task must be completed no later than 12 months prior to the meeting. The Committee is responsible for scientific programs held in cooperation with other organizations. The Committee is required to file a formal written summary annually with the Council.

10) Meetings Committee. The Meetings Committee shall be composed of the Scientific Program Chair of that year, one ex-officio member from the Council, and three appointed members who will serve for three years, one being appointed each year by the President. The senior appointed member will chair the committee. Working closely with the Scientific Program Chair, the Chair of the Meetings Committee shall be responsible for fundraising for the Annual Meeting of that year, for publicity relating to that meeting, and for coordinating all activities relating to the financing of that meeting. The members of the Committee will assist the Chair in fundraising and in executing publicity initiatives. The Chair of the Meetings Committee shall also serve as a member of the Finance Committee.

11) Education Committee. The Education Committee shall be composed of an Education Committee Chair, who shall be appointed by the President for a term of two years (renewable once), and a Curriculum Development Chair, who shall be appointed by the President for a term of three years. These Chairs, with the consent of the President, may appoint additional Committee members as required to institute Society initiatives in Education.

ARTICLE IX (Amendments)

Amendments to the Bylaws shall be initiated according to the same procedure as amendments to the Constitution, except that a majority vote at the annual business meeting shall suffice for ratification.

MEMBERSHIP DIRECTORY

Alphabetical List of Members

Letters in parentheses after each name identify member status,
C = Corporate E = Emeritus H = Honorary M = Individual
ED = Editorial Board S = Student

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